US009468660B2

(12) **United States Patent**
Podbilewicz et al.

(10) **Patent No.: US 9,468,660 B2**
(45) **Date of Patent: Oct. 18, 2016**

(54) **ANTINEMATODAL METHODS AND COMPOSITIONS**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 153 days.

(21) Appl. No.: **13/982,996**

(22) PCT Filed: **Jan. 31, 2012**

(86) PCT No.: **PCT/IL2012/000054**

§ 371 (c)(1),
(2), (4) Date: **Sep. 3, 2013**

(87) PCT Pub. No.: **WO2012/104837**

PCT Pub. Date: **Aug. 9, 2012**

(65) **Prior Publication Data**

US 2013/0336939 A1 Dec. 19, 2013

Related U.S. Application Data

(60) Provisional application No. 61/438,274, filed on Feb. 1, 2011.

(51) **Int. Cl.**

A01N 63/00	(2006.01)
A61K 36/00	(2006.01)
C07K 14/435	(2006.01)
A61K 35/12	(2015.01)
A61K 35/57	(2015.01)
A61K 35/76	(2015.01)

(52) **U.S. Cl.**

CPC ***A61K 36/00*** (2013.01); ***A01N 63/00*** (2013.01); ***A61K 35/12*** (2013.01); ***A61K 35/57*** (2013.01); ***A61K 35/76*** (2013.01); ***C07K 14/4354*** (2013.01); ***C07K 14/43545*** (2013.01)

(58) **Field of Classification Search**

CPC C07K 14/4354
USPC 424/93.2
See application file for complete search history.

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(57) **ABSTRACT**

There are provided methods and compositions useful in cell-cell fusion using Fusion Family (FF) proteins of nematode origin. There are further provided antinematodal methods and compositions, utilizing fusogenic proteins of the nematode Fusion Family.

2 Claims, 15 Drawing Sheets

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Fig. 1A

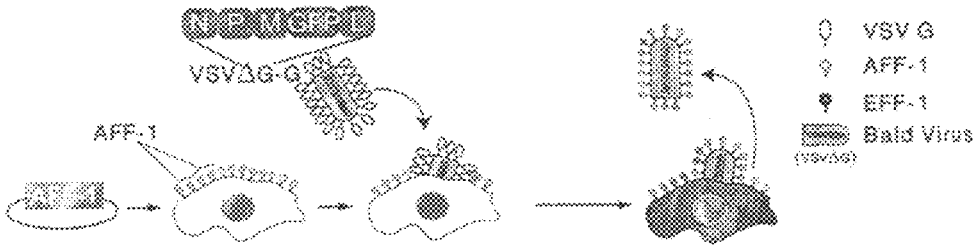


Fig. 1B

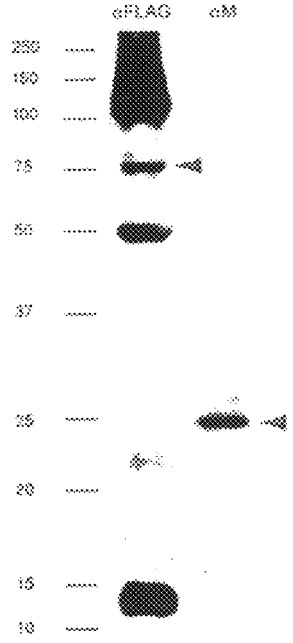


Fig. 1C

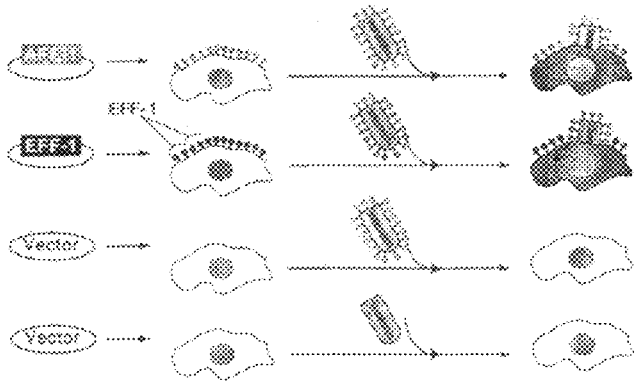


Fig. 1D

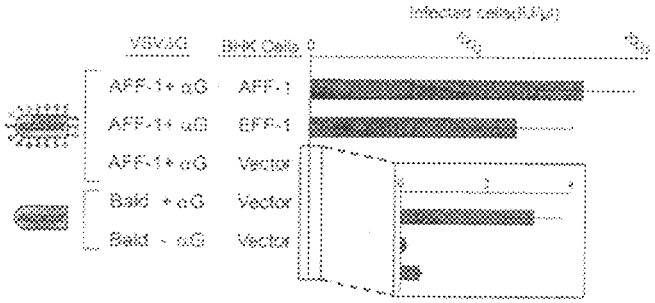


Fig. 1E

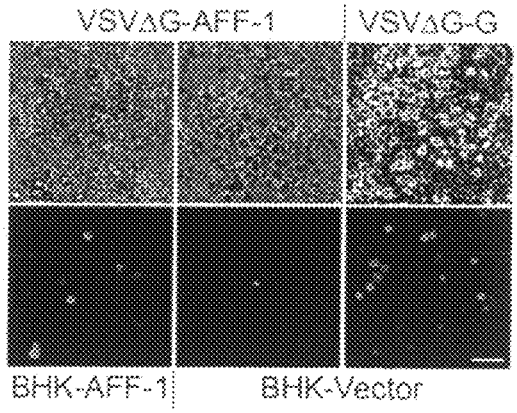
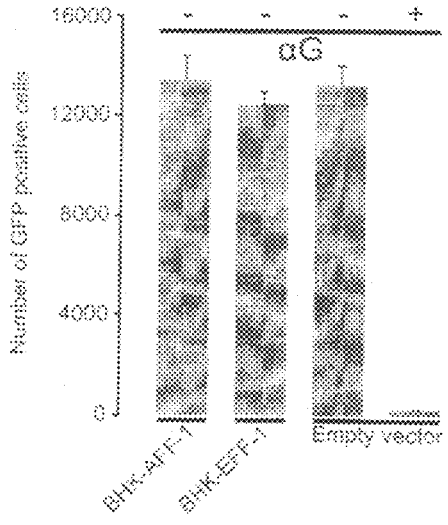


Fig. 1F



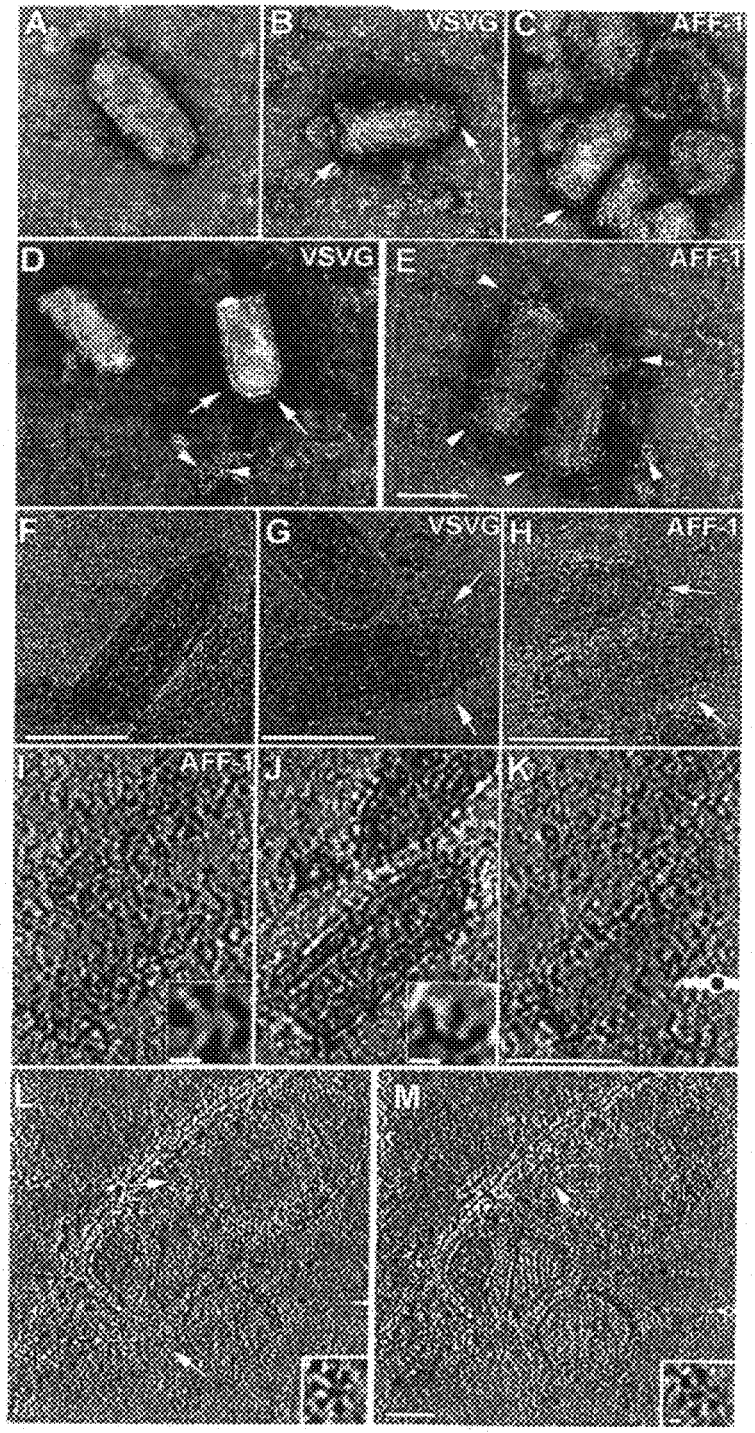
Figs. 2A-C

Figs. 2D-E

Figs. 2F-H

Figs. 2I-K

Figs. 2L-M



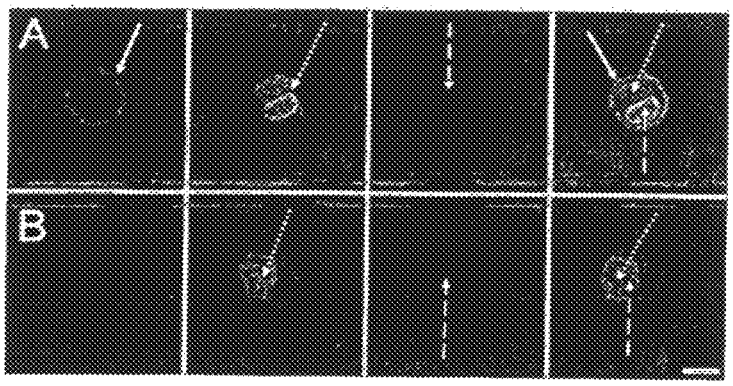

A
B

FIG. 2N

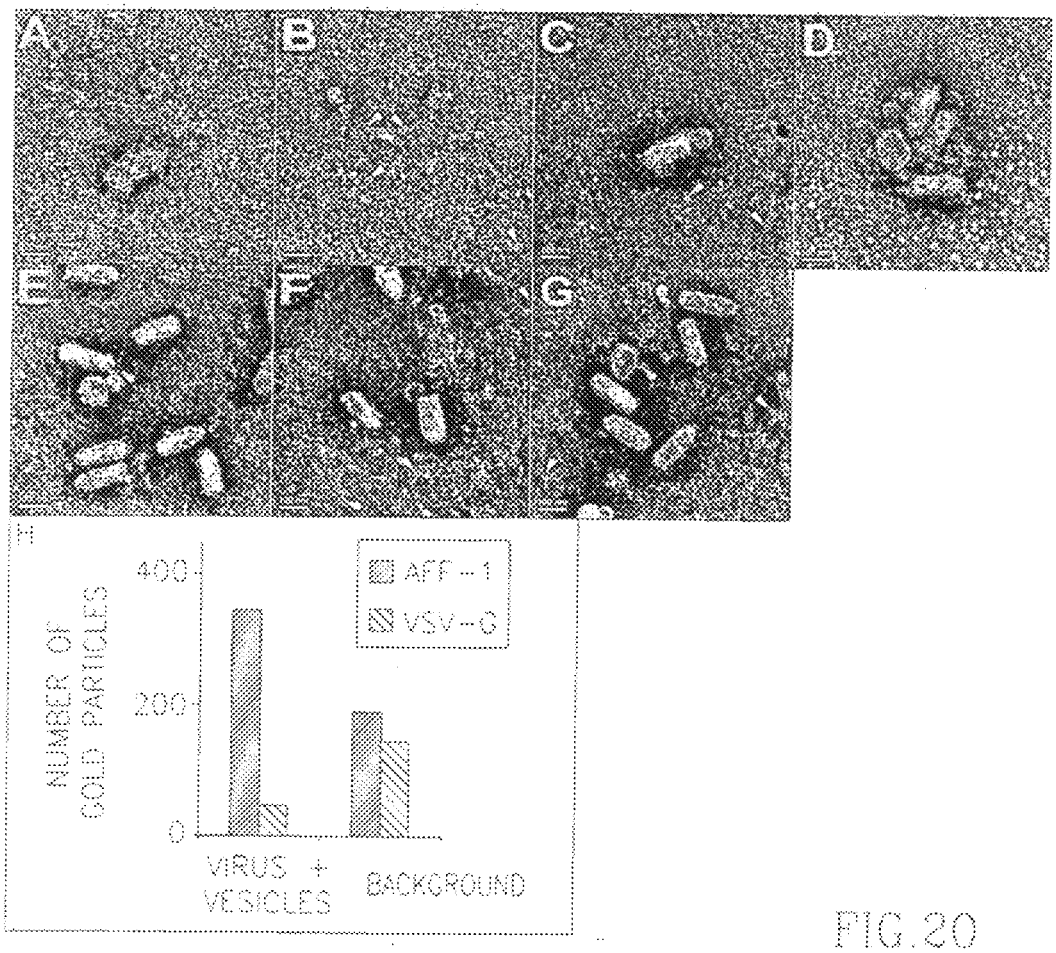
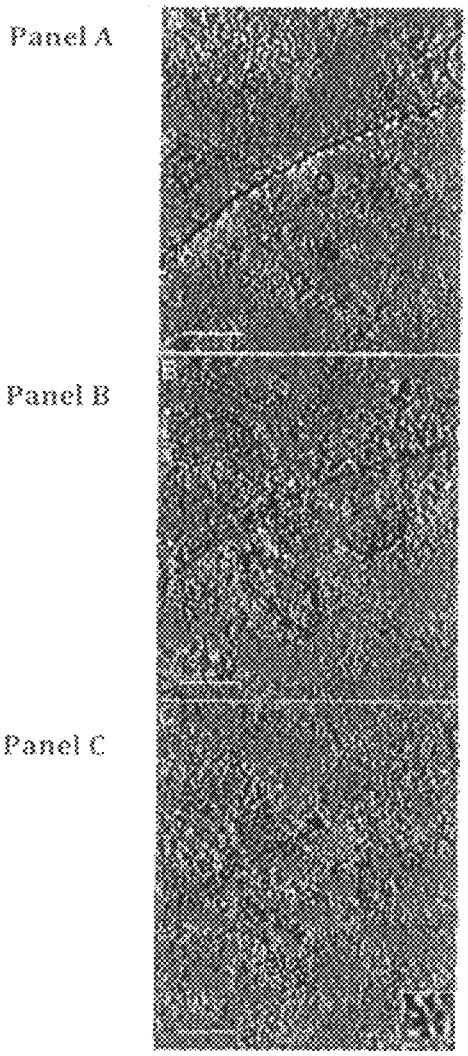

A
B
C
D
E
F
G
H
400
200
0
NUMBER OF GOLD PARTICLES
AFF-1
VSV-G
VIRUS + VESICLES
BACKGROUND

FIG. 2O

Fig. 2P



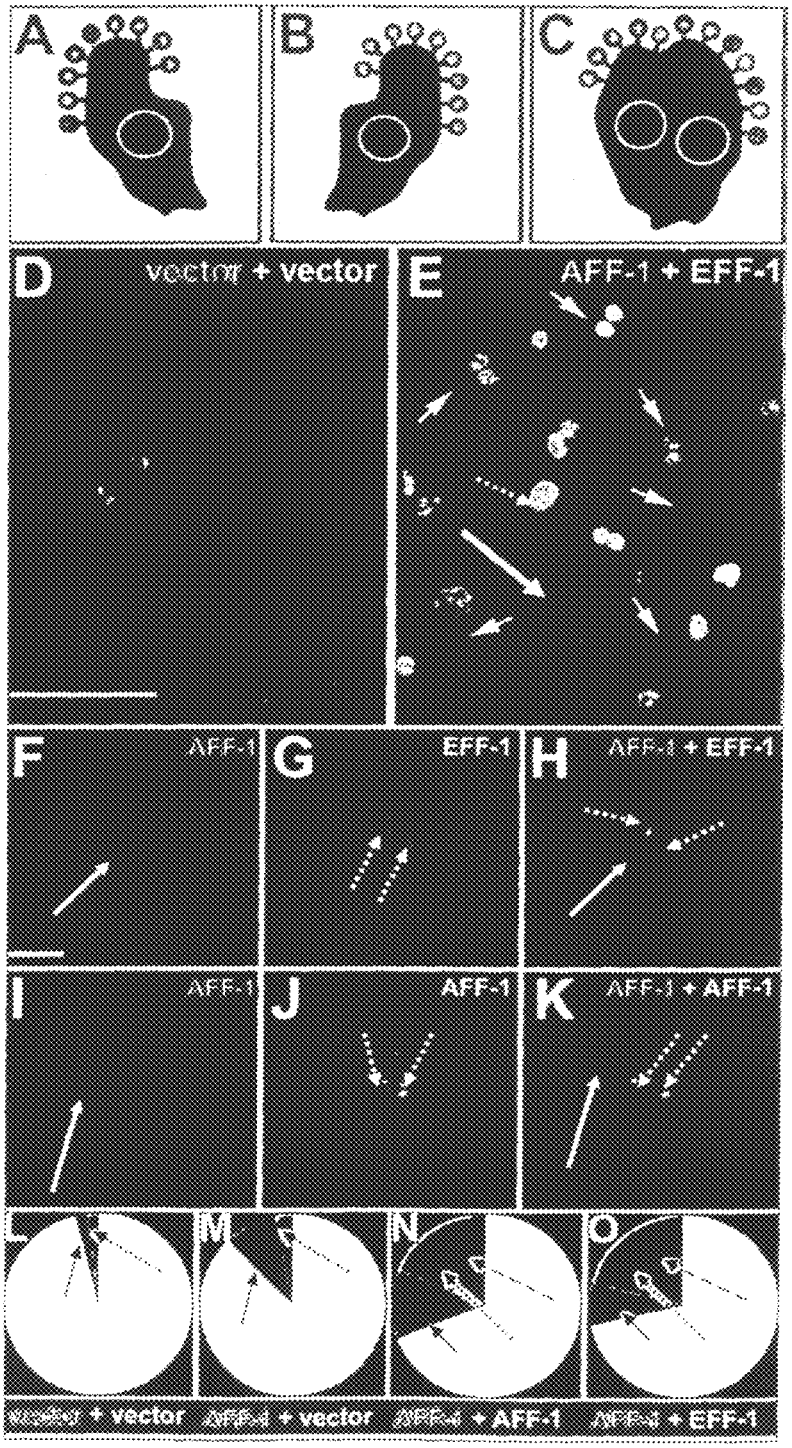

A
B
C
D
vector + vector
E
AFF-1 + EFF-1
F
AFF-1
G
EFF-1
H
AFF-1 + EFF-1
I
AFF-1
J
AFF-1
K
AFF-1 + AFF-1
L
M
N
O
vector + vector
ΔAFF-1 + vector
ΔAFF-1 + AFF-1
ΔAFF-1 + EFF-1

FIG.3A–O

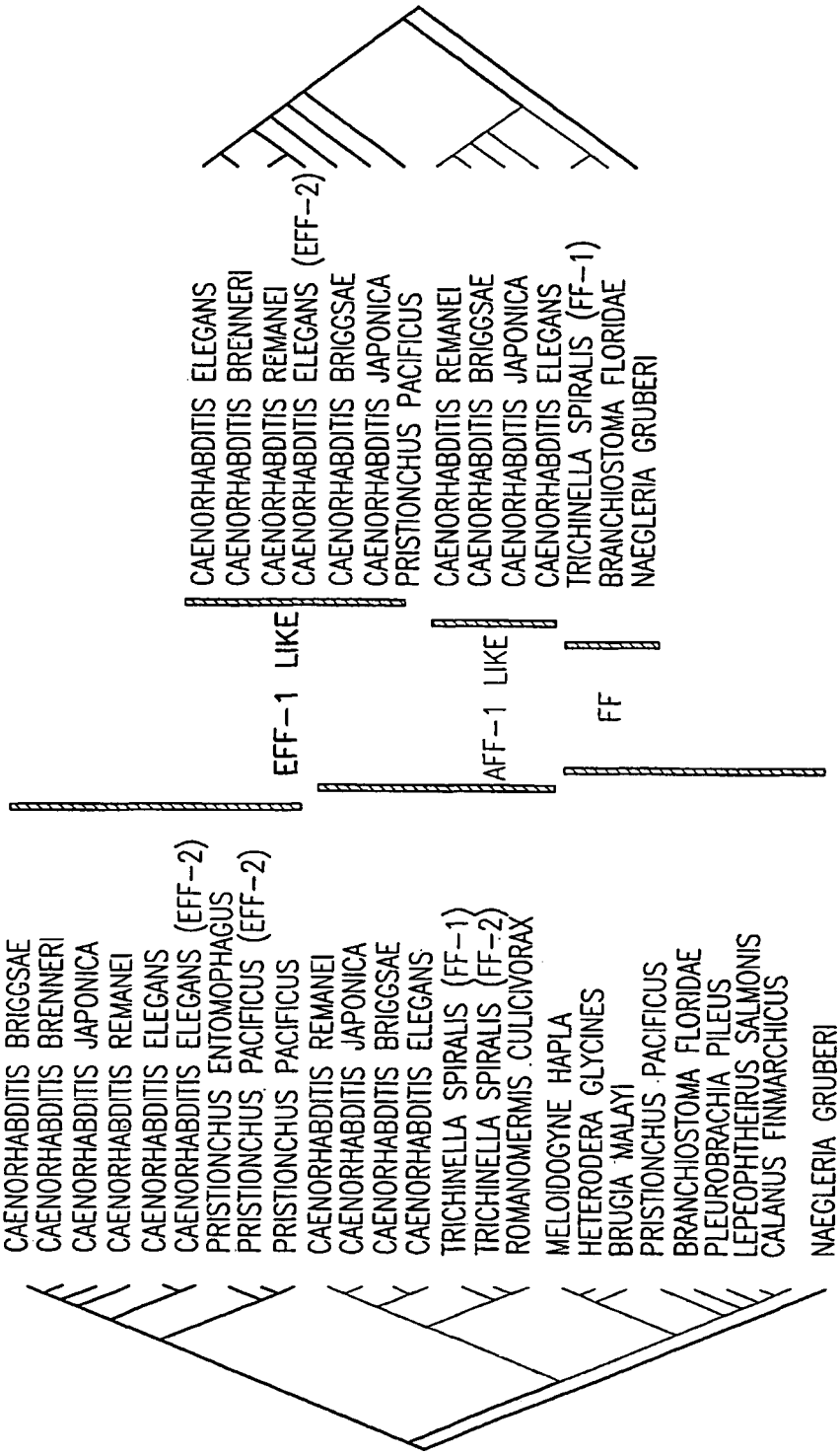

CAENORHABDITIS BRIGGSAE
CAENORHABDITIS BRENNERI
CAENORHABDITIS JAPONICA
CAENORHABDITIS REMANEI
CAENORHABDITIS ELEGANS
CAENORHABDITIS ELEGANS (EFF-2)
PRISTIONCHUS ENTOMOPHAGUS
PRISTIONCHUS PACIFICUS (EFF-2)
PRISTIONCHUS PACIFICUS
CAENORHABDITIS REMANEI
CAENORHABDITIS JAPONICA
CAENORHABDITIS BRIGGSAE
CAENORHABDITIS ELEGANS
TRICHINELLA SPIRALIS (FF-1)
TRICHINELLA SPIRALIS (FF-2)
ROMANOMERMIS CULICIVORAX
MELOIDOGYNE HAPLA
HETERODERA GLYCINES
BRUGIA MALAYI
PRISTIONCHUS PACIFICUS
BRANCHIOSTOMA FLORIDAE
PLEUROBRACHIA PILEUS
LEPEOPHTHEIRUS SALMONIS
CALANUS FINMARCHICUS
NAEGLERIA GRUBERI
EFF-1 LIKE
AFF-1 LIKE
FF
CAENORHABDITIS ELEGANS
CAENORHABDITIS BRENNERI
CAENORHABDITIS REMANEI (EFF-2)
CAENORHABDITIS ELEGANS
CAENORHABDITIS BRIGGSAE
CAENORHABDITIS JAPONICA
PRISTIONCHUS PACIFICUS
CAENORHABDITIS REMANEI
CAENORHABDITIS BRIGGSAE
CAENORHABDITIS JAPONICA
CAENORHABDITIS ELEGANS
TRICHINELLA SPIRALIS (FF-1)
BRANCHIOSTOMA FLORIDAE
NAEGLERIA GRUBERI

FIG.4A

Figs. 4B-E

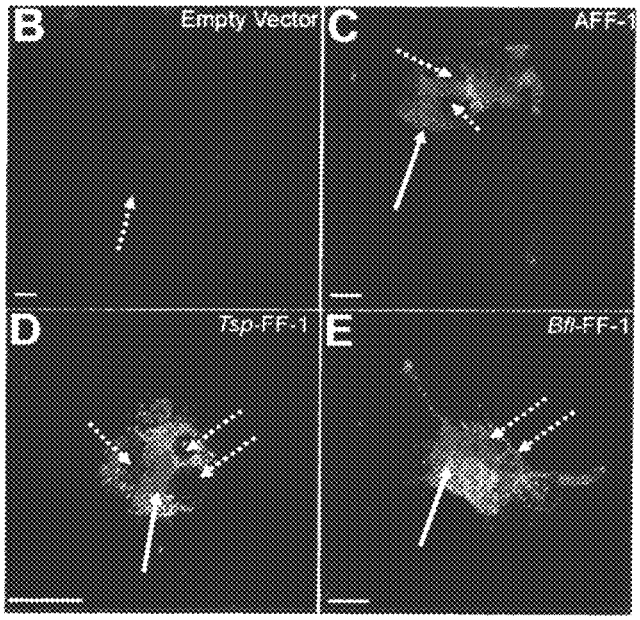
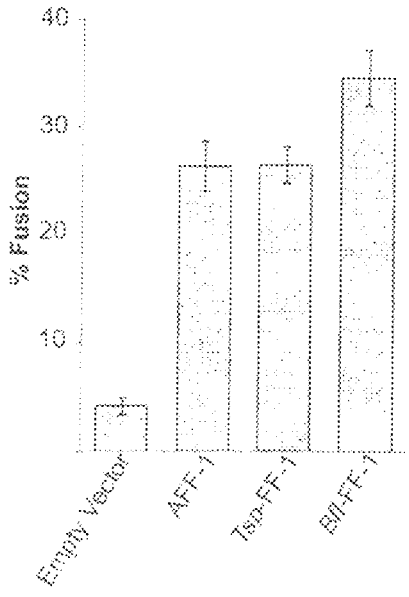


Fig. 4F



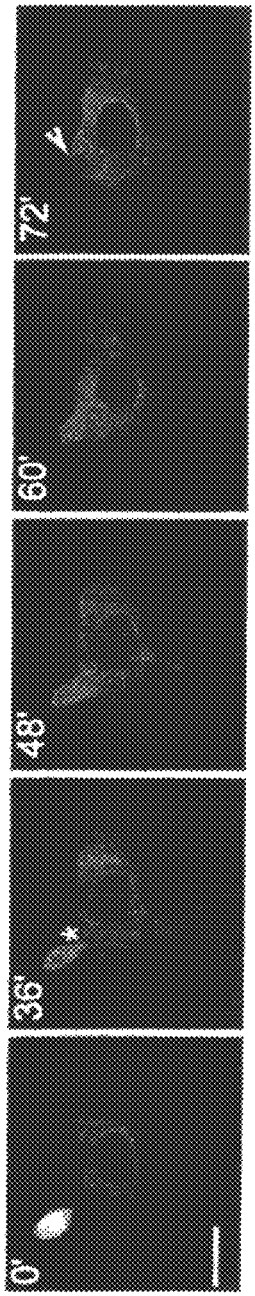


Fig. 5

Fig. 6A


I
II
III
IV
V
VI
VII
AFF-1
EFF-1A
EFF-1B
EFF-1C
EFF-1D
EFF-2
Bb-FF-1
Bb-FF-2
Tsp-FF-1
Pva-FF-1
Ngr-FF-1
Alternative Splicing
Paralogs
Orthologs

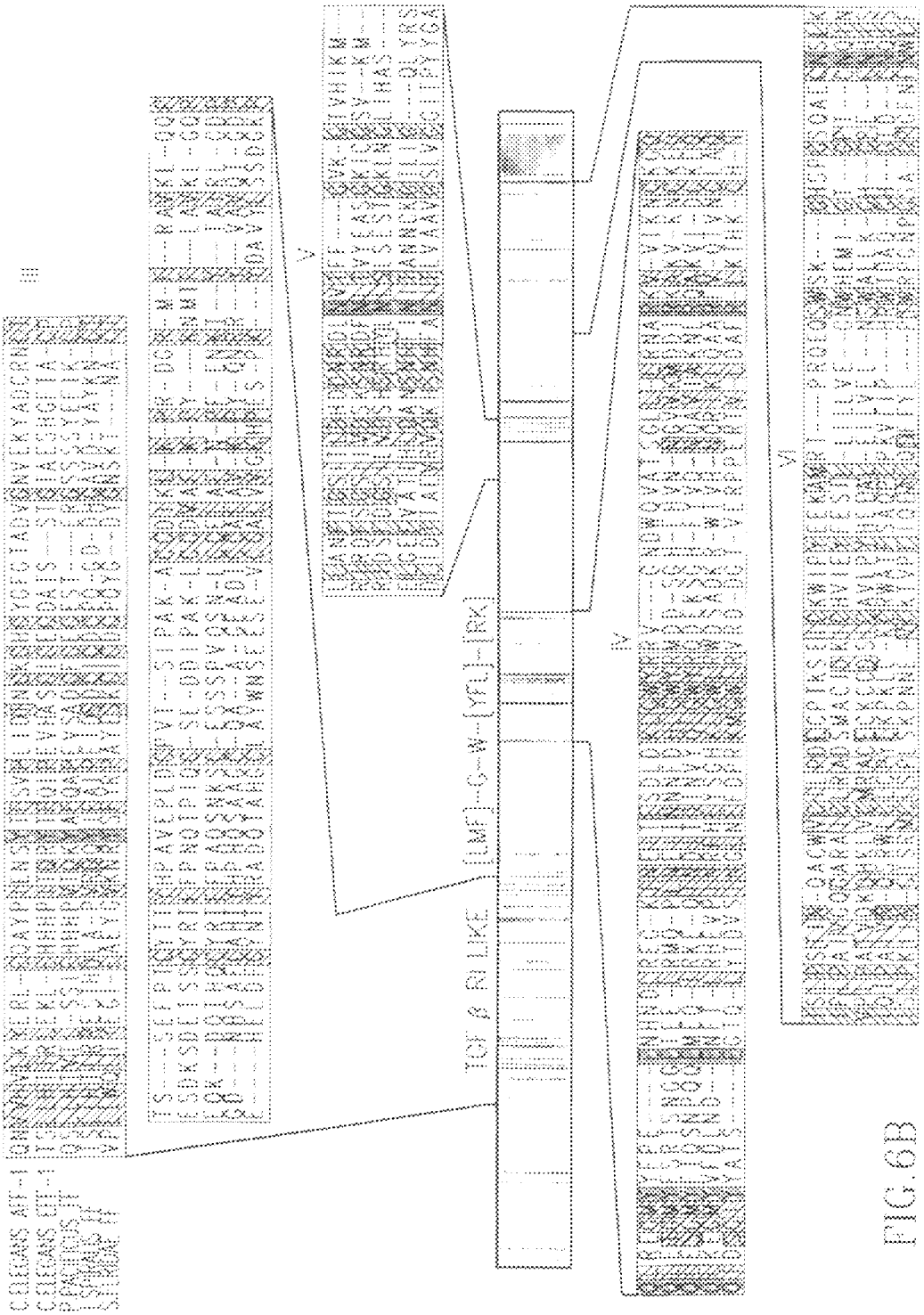
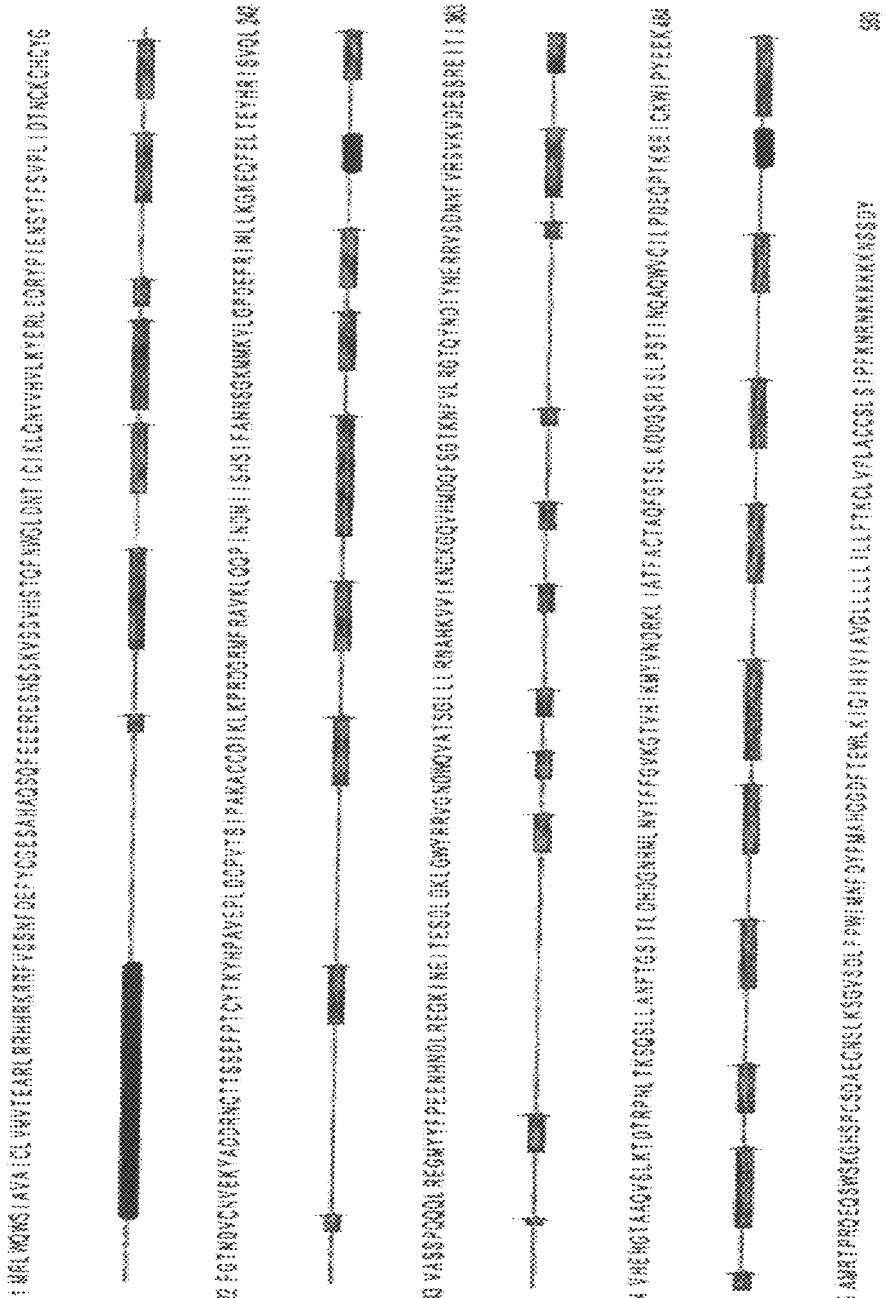
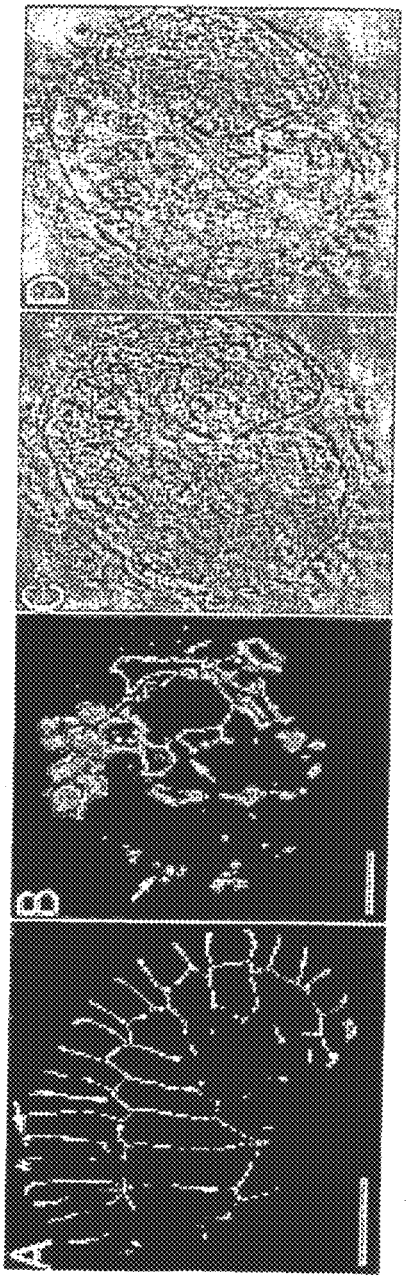


FIG. 6B

Fig. 6C





Figs. 7A-D

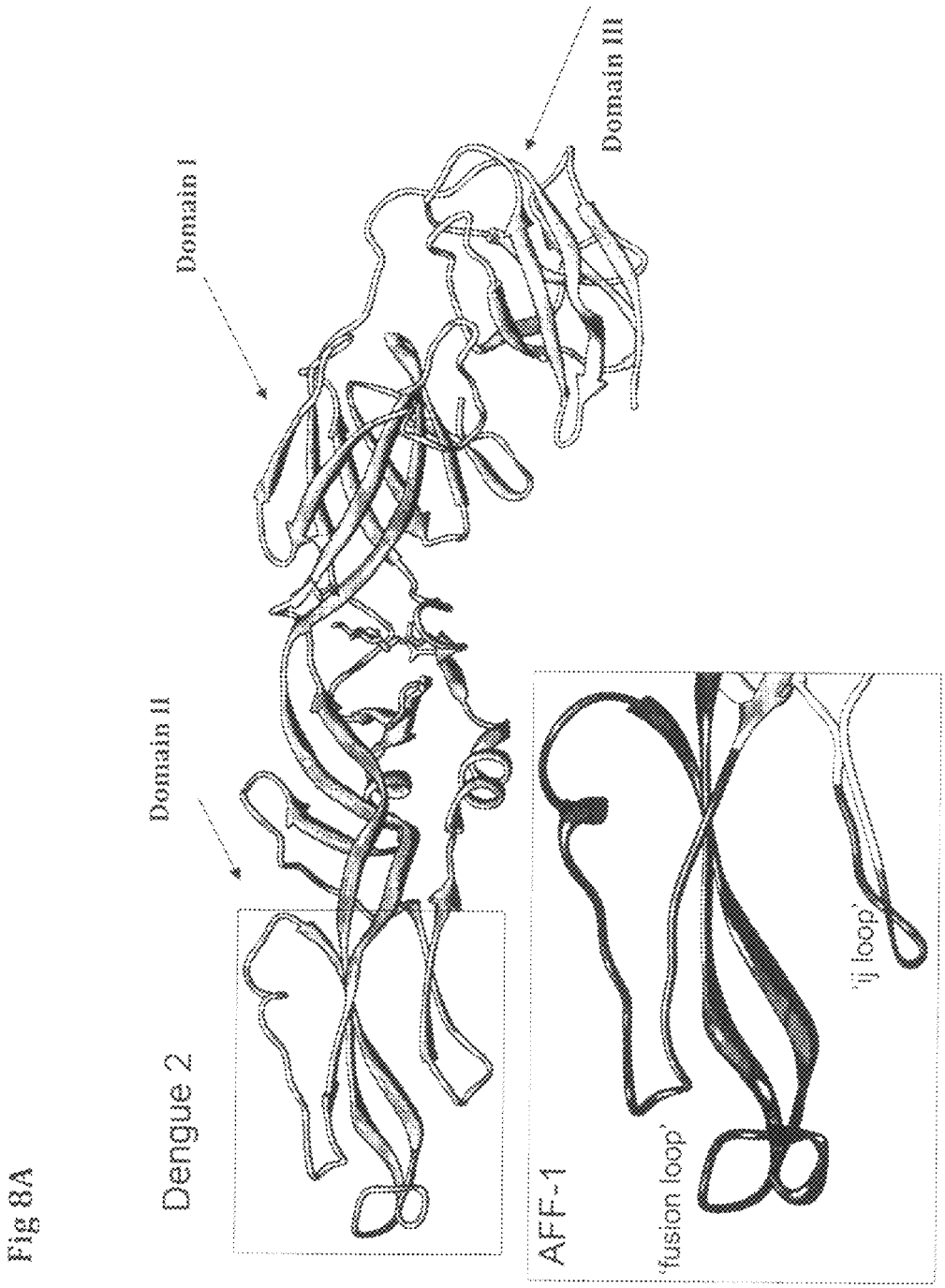

Domain III
Domain I
Domain II
Dengue 2
AFF-1
'fusion loop'
'j loop'

Fig 8A

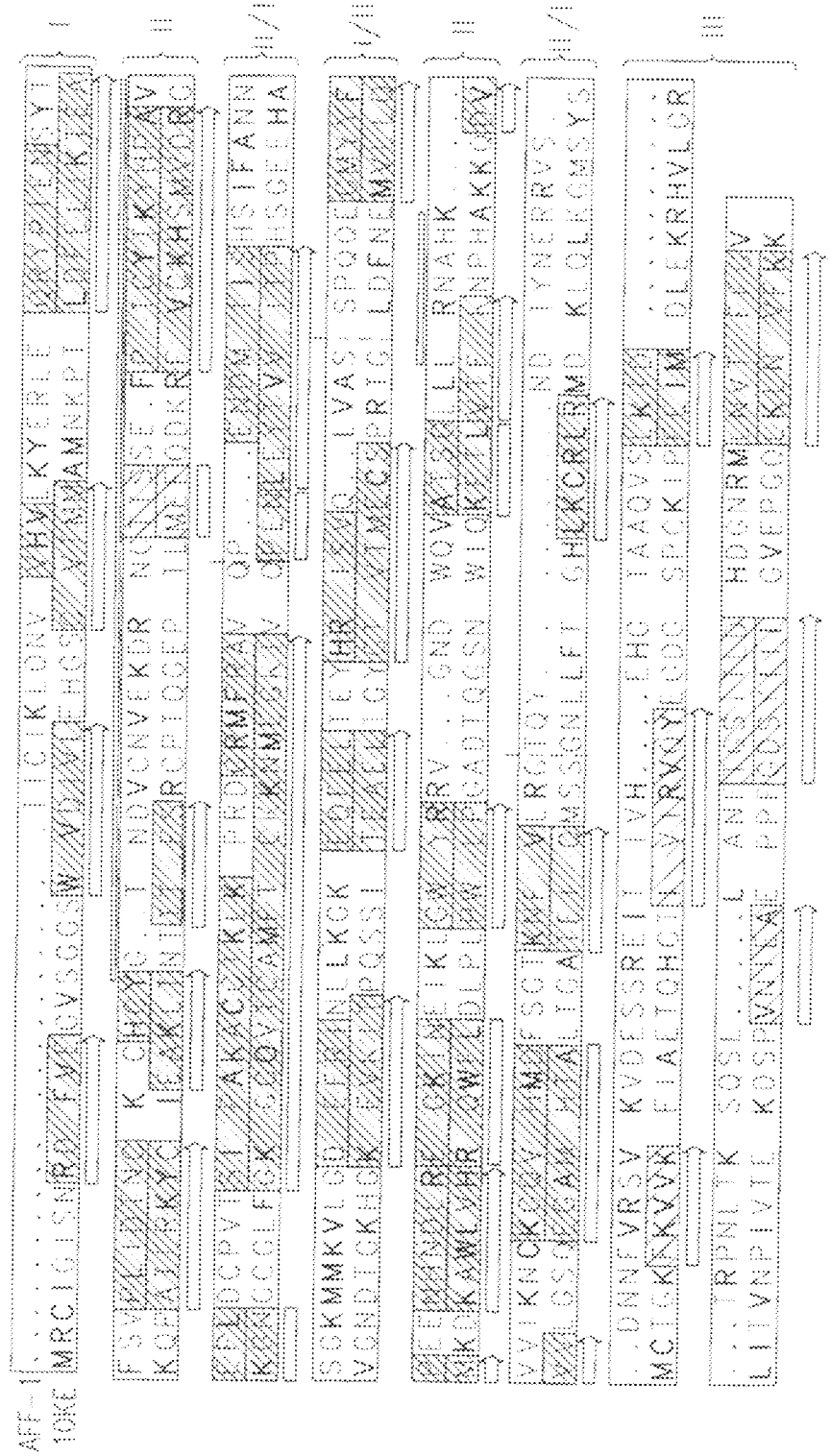


FIG.8B

ANTINEMATODAL METHODS AND COMPOSITIONS

RELATED APPLICATION DATA

This application is the U.S. National Stage of International Application No. PCT/IL2012/000054, filed Jan. 31, 2012, which claims the benefit of U.S. Provisional Application No. 61/438,274, filed Feb. 1, 2011, the contents of each of which are herein expressly incorporated by reference for all purposes.

GOVERNMENTAL SUPPORT OF APPLICATION

This invention was made with government support under AI022470 awarded by the National Institutes of Health. The government has certain rights in the invention.

INCORPORATION-BY-REFERENCE OF MATERIAL ELECTRONICALLY FILED

Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 54,715 byte ASCII (text) file named "Seq_List" created on Jul. 31, 2013.

FIELD OF THE INVENTION

The present invention relates to methods and compositions useful in cell-cell fusion using Fusion Family proteins of nematodes. There are further provided antinematodal methods and compositions, utilizing fusogenic proteins of the nematode Fusion Family.

BACKGROUND OF THE INVENTION

Virtually all membranes can fuse, ranging from small intracellular vesicles and organelles to entire cells. Consequently, membrane fusion is critical for many biological processes such as fertilization, embryonic and postembryonic development, intracellular trafficking and viral infection (1-6). Exoplasmic cell fusion process involves the merger of plasma membranes. This process can be either transient, as in the case of sperm-egg fusion, resulting in a diploid cell that continues to divide, or permanent, resulting in the formation of syncytia multinuclear cells. Such syncytia serve as essential components of several somatic tissues in metazoans, including the myotubes in muscle formation, osteoclasts in bone formation and syncytial trophoblasts in the formation of the mammalian placenta. Exoplasmic cell fusion also takes place during specific viral infections, as enveloped viruses (such as, for example, influenza, HIV and rabies) fuse their membrane with the host's plasma or endosomal membrane. Similar to exoplasmic cell-cell fusion, viral-cell fusion takes place between the external layers of the fusing membranes and, as such, differs in many aspects from endoplasmic fusion events that occur within a cell (for example, vesicular membrane transport between organelles).

Existing models of the molecular mechanisms of membrane fusion rely on experimental and biophysical analyses performed mainly on viral and intercellular fusion-mediating proteins (known as fusogens). However, how well these models correspond to the mechanisms of action of cell-cell fusogens is unknown (4, 5). For example, U.S. Pat. No. 7,402,409 is directed to cell fusion method. Another cell fusion method is described, for example, by Gottesman et. al. (18).

AFF-1 (Anchor-cell Fusion Failure-1) and EFF-1 (Epithelial Fusion Failure-1) proteins from the nematode *C. elegans* are the first identified and therefore the founding members of a family of fusogens (that is, proteins mediating cell to cell fusion through fusion of the lipid bi-layers of the cells), conserved in nematodes (4). The *C. elegans* FF proteins (CeFF's) were shown to induce fusion in heterologous insect cells (for example, references 7-11). aff-1 and eff-1 mutants are viable, but have severe body deformities and reproductive defects associated with cell fusion failure (9,10). EFF-1's function as a fusogen requires its expression in both fusion partners (8). The Fusion Family (FF) family of proteins is very well conserved among nematodes. FF members were identified in various nematode species, suggesting that the FF family is conserved in the phylum Nematoda (4). Only a few members of the FF family have been identified outside nematodes, none of them in plants or in vertebrates.

Nematodes are the most diverse phylum of pseudocoelomates, and one of the most diverse of all animals. Over 28,000 Nematode species have been described (12) and about 16,000 of the nematodes are parasitic. The nematodes have adapted to nearly every, known ecosystem.

Infection by nematodes in general and parasitic nematodes in particular may affect various hosts, such as, for example, livestock, humans, marine habitats, plants, and the like (13), resulting in health-related and financial consequences. Thus, effective control of infection would contribute significantly to agriculture, farming and medicine with a resultant financial implication. For example, the World Health Organization estimates that at least two billion people are infected by parasitic nematodes, while damage by plant parasitic nematodes is estimated at ~4-10 billion $ per year in losses in the U.S and over $80 billion per year in losses worldwide. Antinematodal agents (also known as antihelminthics, anhelmintics and vermicides), currently in use include mostly chemicals, pharmaceuticals or naturally occurring compounds that are designed to kill the parasite or expel it from its host. Nevertheless, most of these antinematodal agents are extremely toxic and if used in improper dosages are dangerous to humans. Furthermore, the continuous use of chemicals leads to the accumulation of resistant worms and inevitably to treatment failure. In addition, controlling pathogens such as parasitic nematodes can be extremely expensive.

Thus, there is an unmet need for new antihelminthic methods and compositions, that are specific, safe, non toxic, inexpensive and with minimal effect on the environment. The use of nematodal fusogens as exogenously expressed mediators for fusion of virus particles to cells or cell-cell fusion between cells of higher organisms of the plant and animal kingdom is neither taught nor suggested in the art.

SUMMARY OF THE INVENTION

The present invention provides methods and compositions for the fusion of cells including but not limited to mammalian cells, plant cells, avian cells, and the like, as well as fusion of cells with viral particles, using fusogenic proteins comprising at least the extracellular portion of a fusogen of nematode origin. The present invention further discloses antinematodal methods and compositions, methods for their preparation, and uses thereof.

The invention is based in part on the unexpected and surprising finding that conserved eukaryotic fusogens, such as, nematode fusogenic proteins of the Fusion Family proteins, can mediate fusion of viral envelopes to cells, by replacing the endogenous viral fusogenic protein(s). The invention is further based in part on the unexpected finding that the Fusion Family (FF) proteins are a family of membrane fusogens that may be interchangeable between species and even beyond the nematode phylum and hence homologs of the family can be used for fusion of non-insect cells, when expressed on the membranes of those cells. Such findings are surprising and unexpected since induced fusion in heterologous insect cells mediated by *C. elegans* FF proteins, does not indicate nor suggest that such fusion proteins are capable of replacing an endogenous viral fusogenic protein(s) or that the FF proteins are interchangeable between species and can be used for fusion of cells of higher, non-insect organisms.

According to some embodiments, a method is provided for a specific, protein mediated cell to cell fusion. The protein which mediates the fusion is a fusion protein, for example, of nematode origin, that is expressed on the surface of the cells and thereby allows/induces/mediates fusion of the cells. In some embodiments, the same fusogenic protein is expressed on the surface of both a first and a second cell. In some embodiments, each of the cells expresses a different fusion protein, both fusogenic proteins belong to the same family of fusogenic proteins (homotypic). In some embodiments, the fusogenic proteins are endogenously expressed proteins. In some embodiments, for at least one of the cells to be fused, the fusogenic protein is an exogenous protein. In some embodiments, the cells are of similar origin. For example, both cells may be mammalian cells. In some embodiments, the cells are of different origin. In some exemplary embodiments, the first cell is a pseudotyped enveloped virus and the other (second) cell is of nematode origin. In other exemplary embodiments, the first cell is a pseudotyped enveloped virus and the other (second) cell is of mammalian or plant origin. In some embodiments, at least one of the cells to be fused is not an insect cell. In some embodiments, the cells are of the higher animal or plant kingdom. Each possibility is a separate embodiment.

According to some embodiments, there is provided a method for fusing a first cell and a second cell to produce a fused, hybrid cell, the method comprising mixing/incubating/placing a first cell comprising a first exogenous nematode fusogenic protein with a second cell comprising a second exogenous nematode fusogenic protein; thereby fusing the first and the second cell. In some embodiments, the first cell and the second cell are of the same origin. In some embodiments when the first cell and the second cell are of the same origin, the cells are not insect cells (i.e. non-insect cells). In some embodiments, the first cell and/or the second cell are non-insect cells. In some embodiments, the first cell and the second cell are of the different origin. In some embodiments, the cells are selected from: virus (virus particle), plant cell, avian cell, animal cells or human cell. Each possibility is a separate embodiment. In some embodiments, the cells are non-insect cells. In some embodiments, the first fusogenic protein and the second fusogenic protein are identical. In other embodiments, the first fusogenic protein and the second fusogenic protein are different. In further embodiments, the first fusogenic protein and the second fusogenic protein are selected from AFF-1, EFF-1 or homologs thereof. In some embodiments, the expression of the first exogenous nematode fusogenic protein in the first cell and/or the second cell is transient. In some embodiments, the expression of the first exogenous nematode fusogenic protein in the first cell and/or the second cell is stable. In some embodiments, when the first cell and the second cell are of the same origin, the cells are not of nematode origin.

According to further embodiments, the method for the specific, protein mediated, cell to cell fusion may further be used for immunotherapy methods and vaccine production by fusing antigen-presenting cells to other cells, wherein both cells express a Fusion Family protein of a nematode origin.

According to further embodiments, the method for the specific, protein mediated, cell to cell fusion may further be used for the production of monoclonal antibodies by fusing cells to generate hybridomas using a physiological and less toxic alternative than currently used methods.

According to further embodiments, the method for the specific, protein mediated, cell to cell fusion may further be used for the study of the mechanism of cell-cell fusion in the field of cancer and stem cell research and therapy.

According to some embodiments, there is provided a composition comprising a non-insect cell or viral particle expressing an exogenous nematode fusogenic protein. In some embodiments, the non-insect cell is selected from mammalian, avian, and plant cells. In further embodiments, the fusogenic protein is capable of mediating fusion of the cell or viral particle to a second cell expressing a second nematode fusogenic protein. In other embodiments, the second cell is selected from a mammalian, an avian, and a plant cell exogenously expressing a second nematode fusogenic protein, or a nematode cell endogenously expressing a nematode fusogenic protein. According to yet further embodiments, the second nematode fusogenic protein is the same or different from the fusogen of the non-insect cell. Each possibility is a separate embodiment.

According to some embodiments, there is provided a method for fusing a first cell and a second cell, the method comprising: incubating a first cell comprising a first exogenous nematode fusogenic protein with a second cell comprising a second exogenous nematode fusogenic protein; thereby fusing the first cell and the second cell to form a fused cell, wherein at least one of the cells is not of insect origin. In some embodiments, the first cell and the second cell are of the same origin. In other embodiments, the first cell and the second cell are of different origin. In some embodiments, the cells are selected from, plant, avian, animal, human, and viral particle. In additional embodiments, the first fusogenic protein and the second fusogenic protein are identical. In other embodiments, the first fusogenic protein and the second fusogenic protein are different. In some embodiments, the first fusogenic protein and the second fusogenic protein are selected from AFF-1, EFF-1 or homologs thereof. In other embodiments, the expression of the first exogenous nematode fusogenic protein in the first cell and/or the second cell is transient. In some embodiments, the expression of the exogenous nematode fusogenic protein in the first cell and/or the second cell is stable.

According to additional embodiments, the method for the specific, protein mediated cell to cell fusion, may be used to specifically target nematodes. The method includes fusing a nematode cell with a viral particle expressing a nematode fusogenic protein. The specific fusion of the viral particle and the nematode cell may lead to a desired effect on the nematode cell, wherein the effect may be achieved without the use of an additional antinematodal agent. A desired effect on the nematode cell, may include, for example, killing the cell, inhibiting growth of the cell, stunning the cell, and the

like. In some embodiments, the mere fusion of cells may lead to the death of the cells. Each possibility is a separate embodiment.

According to further embodiments, the method for the specific, protein mediated, cell to cell fusion, may be used to specifically target antinematodal agents to nematodes. In such embodiments, fusion of the cells is dependant on the expression of a fusogenic protein of nematode origin in the membranes of both fusing cells, wherein the fusogenic proteins may be identical or different. In some exemplary embodiments, one (first) cell is of nematode origin (endogenously expressing the fusion protein) and the other (second) cell is of different origin (such as, of plant origin, mammalian origin, avian origin, insect, enveloped pseudovirus, or the like), wherein the other (second) cell exogenously expresses a fusogenic protein of nematode origin. The other (second) cell may comprise one or more antinematodal agents, such as, for example: a chemical compound (such as, for example, but not limited to: organophospates, carbamates, imidazole derivatives, such as, for example, benzimidazole, Levamisole, Fumigant nematicides, macrolides, avermectin, milbemycin, tetanus toxin, and the like); a nucleic acid (such as, for example, antisense DNA molecules directed against nematode genes; siRNA or other dsRNA molecules directed against nematode genes, and the like); proteins (such as, for example, limited to: an enzyme capable of cleaving a nematode protein, an antibody directed against a nematode protein, a toxin, and the like), or combinations thereof. Upon the specific fusion of the cells, the antinematodal agent may exert an effect on the nematode. The effect may be, for example, kill, stun, and/or inhibit growth of the nematode.

According to further embodiments, the method for the specific, protein mediated, cell to cell fusion may thus be used for the treatment of parasitic nematode infections of plants, animals and humans.

According to some embodiments, there is provided a composition comprising a cell expressing an exogenous nematode fusogenic protein capable of mediating specific fusion of the cell to the nematode cell; wherein the cell comprises an antinematodal agent. The compositions may be used for killing a nematode cell. In some embodiments, the cell may be selected from a mammalian cell, (including any stem cell), avian cell, virus, and plant cell. The exogenous nematode fusogenic protein may be selected from AFF-1, EFF-1 and homologs thereof.

In some embodiments, the antinematodal agent may be selected from a chemical substance, a protein, a nucleic acid, a toxin and combinations thereof. In further embodiments, the antinematodal agent may expressed by the cell.

According to additional embodiments, the exogenous nematode fusogenic protein in the cell may be transient. In some embodiments, the expression of the exogenous nematode fusogenic protein in the cell may stable.

According to yet further embodiments, the nematode may be selected from, but not limited to: *Caenorhabditis elegans, Caenorhabditis briggsae, Caenorhabditis japonica, Caenorhabditis ramanei, Caenorhabditis brenneri, Caenorhabditis* sp5,7,9,11, *Trichinella spiralis, Trichinella pseudospiralis, Trichinella papuae, Pristionchus entomophagus, Pristionchus maupasi, Pristionchus pacificus, Meloidogyne incognita, Meloidogyne arenaria, Meloidogyne hapla, Globodera pallida, Ancylostoma caninum, Brugia Malayi, Haemonchus contortus, Ascaris suum, Oscheius tipulae, Dirofilaria immitis, Howardula aoronymphium, Litomosoides sigmodontis, Heterodera glycines, Romanomermis culicivorax, Trichuris muris, Strongyloids ratti, Onchocerca volvulus, Teladorsagia circumcincta, Wuchereria bancrofti*, and *Loa loa.*

According to some embodiments, there is provided a composition comprising a virus comprising/expressing a nematode fusion protein, wherein said fusogenic protein is capable of mediating fusion of the virus to a cell of the nematode; wherein said fusion induces killing of the nematode. In some embodiments, the nematode fusogenic protein is selected from AFF-1, EFF-1 and homologs thereof. The nematode may be selected from, but not limited to: *Caenorhabditis elegans, Caenorhabditis briggsae, Caenorhabditis japonica, Caenorhabditis ramanei, Caenorhabditis brenneri, Caenorhabditis* sp5,7,9,11, *Trichinella spiralis, Trichinella pseudospiralis, Trichinella papuae, Pristionchus entomophagus, Pristionchus maupasi, Pristionchus pacificus, Meloidogyne incognita, Meloidogyne arenaria, Meloidogyne hapla, Globodera pallida, Ancylostoma caninum, Brugia Malayi, Haemonchus contortus, Ascaris suum, Oscheius tipulae, Dirofilaria immitis, Howardula aoronymphium, Litomosoides sigmodontis, Heterodera glycines, Romanomermis culicivorax, Trichuris muris, Strongyloids ratti, Onchocerca volvulus, Teladorsagia circumcincta, Wuchereria bancrofti*, and *Loa loa*. Each possibility is a separate embodiment.

In some embodiments, the virus may be selected from Retrovirus, Hepadnavirus, Poxyvirus, Rhabdoviridae viruses, Paramyxoviruses, Herpes viruses and Coronavirus. Each possibility is a separate embodiment.

According to further embodiments, there is provide a method for the targeted killing of a nematode, the method comprising contacting the nematode with a cell comprising an antinematodal agent, wherein the cell further comprises an exogenous fusogenic protein capable of mediating fusion of the cell and a cell of the nematode; and wherein the fusion induces killing of the nematode. The cell may be selected from mammalian cell, stem cell, avian cell, virus, and plant cell. In some embodiments, the exogenous fusogenic protein is a nematode protein selected from AFF-1, EFF-1 and homologs thereof.

According to additional embodiments, the antinematodal agent may be selected from a chemical substance, a protein, a nucleic acid, a toxin and combinations thereof. The antinematodal agent may be expressed by the cell. Each possibility is a separate embodiment.

According to some embodiments, there is provided a method for the targeted killing of a nematode, the method comprising contacting the nematode with a virus comprising an exogenous nematode fusion protein, capable of mediating fusion of the virus to a cell of the nematode; wherein the fusion induces killing of the nematode. In some embodiments, the exogenous fusogenic protein is a nematode fusogenic protein selected from AFF-1, EFF-1 and homologs thereof. In some embodiments, the nematode may be selected from, but not limited to: *Caenorhabditis elegans, Caenorhabditis briggsae, Caenorhabditis japonica, Caenorhabditis ramanei, Caenorhabditis brenneri, Caenorhabditis* sp5,7,9,11, *Trichinella spiralis, Trichinella pseudospiralis, Trichinella papuae, Pristionchus entomophagus, Pristionchus maupasi, Pristionchus pacificus, Meloidogyne incognita, Meloidogyne arenaria, Meloidogyne hapla, Globodera pallida, Ancylostoma caninum, Brugia Haemonchus contortus, Ascaris suum, Oscheius tipulae, Dirofilaria immitis, Howardula aoronymphium, Litomosoides sigmodontis, Heterodera glycines, Romanomermis culicivorax, Trichuris muris, Strongyloids rata, Onchocerca volvulus, Teladorsagia circumcincta, Wuchereria bancrofti*, and *Loa loa*. The

virus may be selected from retrovirus, Hepadnavirus, Poxyvirus, Rhabdoviridae viruses, Paramyxoviruses Herpes viruses and Coronavirus.

According to yet further embodiments, there is provided a method for the treatment of a nematode infection in a subject, the method comprising administering to the subject a composition comprising a cell expressing an exogenous nematode fusion protein, wherein fusion of said cell and a nematode cell infecting the subject induces death or inhibition of growth of the nematode. In some embodiments, the subject is human. In some embodiments, the subject is an animal. In some embodiments, the administering is selected from oral administration, injection, suppository and topical application. In further embodiments, the cell may further include an antinematodal agent, selected from a chemical substance, a protein, a nucleic acid, a toxin and combinations thereof. In additional embodiments, the cell may be selected from a mammalian cell, stem cell, avian cell, virus, and plant cell.

According to some embodiments, there is provided a transgenic plant stably expressing a fusogenic protein of the nematode family.

According to yet further embodiments, there is provided a viral vector for the expression of a nematode fusogenic protein on the surface of a virus.

According to additional embodiments, there is provided a recombinant cell expressing a polynucleotide encoding a polypeptide comprising an amino acid sequence at least 15% identical to the amino acid sequence of a nematode fusogenic protein. In some embodiments, the cell may be selected from mammalian cell, stem cell, avian cell, virus, and plant cell. In some exemplary embodiments, the nematode fusogenic protein is selected from Ce-AFF-1 (SEQ ID NO: 23), Ce-EFF-1 (SEQ IS NO:24), tsp-FF-1 (SEQ. ID. No. 25) and/or Bfl-FF-1 (SEQ ID No. 26). Each possibility is a separate embodiment.

According to yet additional embodiments, there is provided a composition for killing a nematode cell, the composition comprising: a recombinant cell expressing an exogenous polynucleotide encoding a polypeptide comprising an amino acid sequence at least 15% identical to the amino acid sequence of a nematode fusion protein, wherein said recombinant cell further comprises an anti nematodal agent.

According to some embodiments, there is further provided a kit comprising a composition comprising a recombinant cell expressing a fusogenic protein of nematode origin and; instructions for using said composition for the treatment of nematode infection.

These and additional benefits and features of the invention could better be understood by those skilled in the art with reference to the following detailed description taken in conjunction with the figures and non-limiting examples.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and form a part of the specification, illustrate certain embodiments of the present invention, and together with the description serve to explain the principles of the invention. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than restrictive. The figures are listed below.

FIG. **1**A shows a schematic illustration of the generation of recombinant single round infective VSVΔG-AFF-1 in vitro. BHK cells were transfected with a plasmid encoding aff-1 (Table 2, below) and expressed the protein on the cell surface. Cells were then infected with the G-complemented VSVΔG recombinant virus (VSVΔG-G). The viral genome encodes GFP in place of the fusogenic glycoprotein G. Infection results in viral induced expression of GFP by target cells (gray cytoplasm). VSVΔG-AFF-1 pseudoviruses were harvested from the supernatant.

FIG. **1**B shows a pictograph of Western Blot analysis of purified. VSVΔG-AFF-1 pseudoviruses demonstrating incorporation of AFF-1 into pseudotypes. Left Panel (A) depicts Mouse anti-Flag antibody recognizing bands of an apparent molecular weight (MW) of 75 kDa. The apparent MW of AFF-1 in Sf9 insect cells is 75 kDa (3). The theoretical MW of AFF-1 is 67 kDa (8). The extra bands reflect oligomers, processing and differential glycosylation of AFF-1 in BHK cells. Right panel (B) depicts Mouse anti-VSV M antibody, which identified a protein of an apparent molecular weight of 25 kDa corresponding to the predicted molecular weight of VSV-M (arrow head).

FIG. **1**C shows a schematic illustration summarizing VSVΔG-AFF-1 infection of BHK cells. Cells were transfected with aff-1, eff-1 or vector plasmid and infected with VSVΔG-AFF-1. Cells transfected with empty vector and infected with bald particles served as negative controls.

FIG. **1**D shows bar graphs representing titers of VSVΔG pseudoviruses. The type of protein on the viral membrane (Bald or AFF-1) and on the BHK cell membrane (Vector, AFF-1 or EFF-1) is indicated. Anti-VSVG antibody (ΔG) was used to neutralize any, residual VSVΔG-G virus (shown in FIG. **1**F, below). Titers are in infectious units (IU) representing the number of cells expressing. GFP per microliter 24 hours after virus inoculation. Data are mean+/−SE (n=3 experiments). The inset shows background infection.

FIG. **1**E shows images of infected BHK cells. Infection of BHK cells monitored as GFP expression; phase contrast (top panels) versus fluorescence (bottom panels). Scale bar is 50 μm.

FIG. **1**F shows a bar graph showing VSVΔG-G infection of cells expressing CeFFs and in the presence of anti-VSVG. Cells expressing AFF-1, EFF-1 or transfected with empty vector were infected with VSVΔG-G pseudo typed virus (1.5×10^7 IU). Infection was preformed in the presence (+) or absence (−) of anti G antibodies (αG) (1:100). Cells were examined by FACS (total number of cells counted 20,000 cells) after 24 hours. Transfection/Expression of FF proteins did not affect infection efficiency of VSVΔG-G pseudo typed virus (The two-tailed P value equals 0.49-0.89). αG efficiently blocked infection of VSVΔG-G. Results are presented as mean of three independent experiments+/− standard error.

FIGS. **2**A-P shows Electron Microscopy pictures of various Recombinant VSVΔGs or immunofluorescence of Sf9 cells expressing AFF-1. Negative stained vesicles were obtained from the following viruses: FIG. **2**A bald virus preparations (VSVΔG); FIG. **2**B VSVΔG-G virus preparation; FIG. **2**C—VSVΔG-AFF-1 pseudotype virus preparations. Arrows point to surface particles.

Anti AFF-1 polyclonal antibodies followed by immunogold labeling and negative stain of FIG. **2**D—VSVΔG-G virus preparation, FIG. **2**E—VSVΔG-AFF-1 pseudotype virus preparation.

Cryo TEM: FIG. **2**F≠VSVΔG virus; FIG. **2**G—VSVΔG-G; FIG. **2**H VSVΔG-AFF-1.

FIGS. **2**I-K—show Top (**2**I), Center (**2**J) and bottom (**2**K) slice from VSVΔG-AFF-1 tomogram, respectively.

FIGS. **2**L-M show slices form cryoET of vesicles co-purified with VSVΔG-AFF-1 preparation displaying penta- or hexa-meric "flower" shaped complexes (Arrows). Scale bars are 100 nm and 10 nm for insets; Arrows: surface spike

assemblies; Arrowheads: gold particles; White square: indicating area shown magnified in inset.

FIG. **2**N. Immunofluorescence in Sf9 cells expressing AFF-1-Flag (transfected with 3 μg/ml aff-1 plasmid (Table 2)) with either: Panel A—#8 mouse polyclonal antibodies against AFF-1, diluted 1:500 in TBST or Panel B—pre-immune serum. Secondary antibody—Alexa Fluor 568 goat anti mouse IgG (H+L, Invitrogen Cat#A11004) diluted 1:500 in TBST. Order of pictures in each panel, left to right: Panel A: AFF-1 (white, solid arrow (representing red)); Transfection marker nuclear/cytoplasmic (dashed arrow (representing green)); DAPI, DNA (Long dashed arrow (representing blue)); overly of staining. Panel B; pre-immune serum; Transfection marker nuclear/cytoplasmic (dashed arrow (representing green)); DAPI, DNA (long dashed arrow (representing blue)); overly of staining. Scale bar is 10 μm.

FIG. **2**O (panels A-H) demonstrate quantification of immunogold labeling. Panel A—shows pictograms of immunogold labeled VSVΔG-AFF-1 (asterisk). Panel B shows pictograms of immunogold labeled vesicle isolated from VSVΔG-AFF-1 prep (asterisk) with some background staining (arrowheads). Panel C—shows pictograms of immunogold labeled VSVΔG-AFF-1 (center) with some background staining (arrowheads). Panel D—shows pictograms of immunogold labeled vesicles and VSVΔG-AFF-1 viruses. Panel E—shows pictograms of VSVΔG-G stained with anti AFF-1 (negative control) antibody showing some non specific immunogold labeling (arrowheads). Panel F—shows pictograms of VSVΔG-G (center) with some background staining (arrowheads). Panel G—shows pictograms of immunogold labeled vesicles isolated from VSVΔG-G prep (asterisk) with some background staining (arrowheads). Panel H—shows bar graphs representing number of gold particles (Y axis) recognizing viruses and vesicles versus background of VSVΔG-AFF-1 or VSVΔG-G samples stained with anti-AFF-1. Anti-AFF-1 show specific virus/vesicle recognition of VSVΔG-AFF-1 compared to control VSVΔG-G. The difference in background staining between VSVΔG-AFF-1 and VSVΔG-G grids is not statistically significant (P=0.4588; n=30 quantified images per virus);

FIG. **2**P (panels A-C) shows slices from cryoET of vesicles that co-purified with VSVΔG-AFF-1. Panel A—Top; Panel B—center; Panel C—bottom of slice from cryoET of vesicle preparations displaying penta- or hexameric "flower" shaped assemblies, Scale bars are 100 nm and 10 nm for the inset; white box: magnified area shown in inset.

FIGS. **3**A-O—Fusion of BHK-AFF-1 and BHK-EFF-1 cells. FIGS. **3**A-C are schematic illustrations of experimental design of a color mixing assay. FIG. **3**D is a pictogram showing Negative control. Mixed cells co-transfected with empty vector and a (red) cytoplasmic marker (RFPnes) or a (cyan) nuclear marker (CFPnls) showed no color mixing. Scale bar is 100 μm.

FIG. **3**E is a pictogram showing BHK-AFF-1 expressing cells (solid arrow (representing Red)) and BHK-EFF-1 expressing cells (dashed arrow (representing Cyan)) that were mixed. Hybrids express cyan nuclei and red cytoplasm (as indicated by the solid, short arrow heads).

FIGS. **3**F-H show pictograms of: FIG. **3**F: AFF-1 expressing BHK cells with (red) cytoplasm (represented by solid arrow); FIG. **3**G: EFF-1 expressing BHK cells with (cyan) nuclei (represented by dashed arrow); FIG. **3**H: BHK cells with (red) cytoplasm (represented by solid arrow) surrounding, two (cyan) nuclei (represented by dashed arrows) appeared following expression of AFF-1 in BHK cells, expression of EFF-1 expression in BHK cells and mixing of the cells. (Scale bar is 10 μm.)

FIGS. **3**I-K show pictograms of: FIG. **3**I: AFF-1 expressing BHK cell with (red) cytoplasm (solid arrow, representing red cytoplasm); FIG. **3**J: AFF-1 expressing BHK cell with (cyan) nuclei (dashed arrow, representing cyan nuclei); FIG. **3**K: BHK cell with red cytoplasm (solid arrow, representing red cytoplasm) surrounding cyan nuclei (dashed arrow, representing cyan nuclei), which appeared following mixing of the cells.

FIGS. **3** L-O show quantification of the content mixing experiments in pie graphs, which represent the fraction of multinucleated cells (2 nuclei or higher). Results are mean of four independent experiments (n≥1000 total cells): FIG. **3**L is a pie graph of quantity of cells transfected with Empty vectors. All multinucleated cells are bi-nucleated (total dividing cells 4%); FIG. **3**M is a pie graph of quantity of AFF-1 expressing cells (solid arrow (representing red)) mixed with empty vector transfected cells (dashed arrow (representing cyan)). Elevation in multinucleation was only observed for AFF-1 expressing cells (solid arrow, 11%; dashed arrow, 3%). One cell with a single nucleus expressing both markers (red and cyan) was observed; FIG. **3**N is a pie graph of quantity of AFF-1 expressing cells (solid arrow (representing red)) mixed with AFF-1 expressing cells (dashed arrow (representing cyan) resulting in four cell populations—mononucleated white and multinucleated red (represented by solid arrow, 13%), cyan (represented by dashed arrow, 12%) and mixed (represented by long dashed arrow (purple); 11%); FIG. **3**O is a pie graph of quantity of AFF-1 expressing cells (solid arrow (representing red), 9%) mixed with EFF-1 expressing cells (dashed arrow (representing cyan), 11%). AFF-1 and EFF-1 expressing cells fuse (long dashed arrow (representing purple) 18%).

FIGS. **4**A-F The FF Family of Eukaryotic Cell-Cell Fusogens: FIG. **4**A shows scheme of two trees produced using maximum parsimony analysis. Phylogenic relationships of 25 taxa (left) and 14 taxa (right) based on either the TGFβ-RI like domain or the full length extracellular domain respectively, show classification of FF proteins into three subgroups (EFF-1-like, AFF-1-like and FF). FIG. **4**B-E shows pictograms of immunofluorescence with anti-Flag antibodies (solid arrow (representing green)), and nuclei DAPI staining (dashed arrow (representing blue)) on BHK cells transfected with: FIG. **4**B—empty vector; FIG. **4**C—aff-1; FIG. **4**D—Tsp-ff-1; and FIG. **4**E—Bfl-ff-1. Co-transfection marker (original color—red). The images are representative of hundreds of fields in at least eight independent experiments. Scale bars represent 20 μm. For cells not expressing the construct (no red fluorescence) only the nuclei are visible. FIG. **4**F shows bar graphs illustrating fusion index for transfected BHK cells expressing FF proteins and negative control. Data are means±SE. Empty vector, n=14, aff-1, n=14, Tsp-ff-1, n=8, n=9; n represents number of experiments.

FIG. **5** shows time lapse images of AFF-1-mediated fusion of mammalian cells. BHK cells co-transfected with AFF-1 and pRFPnes (white). The cells fuse as indicated by the diffusion of the marker from the brighter cell (36 min, asterisk) to the larger cell. After 72 minutes the marker is homogenously distributed and excluded from the second nucleus (arrowhead). Scale bar 20 μm. n>3 experiments.

FIGS. **6**A-C shows sequence analysis of FF proteins. FIG. **6**A shows distribution of conserved sequence motifs in FF paralogs and orthologs. Sequence motifs are numbered (color coded): I (representing Green)—Signal peptide (SP);

II (representing Pink)—Pro-domain; III (representing Brown)—TGFβ-RI-like domain; IV (representing Yellow)—"[LMF]-G-W-[YFL]-[RK] motif"; V (representing Cyan)—Putative protein-protein interaction domain; VI (representing Purple)—Membrane proximal stem domain, VII (representing Ocher)—Transmembrane domain (TM). *C. elegans* paralogs are listed by gene name AFF-1, EFF-1, EFF-2 (C26D10.7). EFF-1 Alternative splicing variants (EFF-1 A-D) are also shown. Overall sequence identity to AFF-1 is indicated under the gene name. Local sequence identity to AFF-1 (%) is indicated within each domain. Sequence limits are indicated under the illustration unless it is identical to previously shown schematic as in the case for EFF-1 isoforms. Sequences retrieved from *B. floridae* v2.0 assembly are listed as Bf-FF-1 and FF-3 which correspond to protein model id's 104514 and 104513 respectively. *T. spiralis* (Tsp FF-1) and *P. pacificus* (Ppa FF-1) correspond to gi|162730680 and Contig235.2 of the PpaFreeze1.bases database. Sequences retrieved for *N. gruberi* (Ngr-FF-1) correspond to gi|284087402 (Table 4). Accession numbers/database identifier of the various sequences are listed in Table 4. Annotation was preformed as previously described (2). FIG. **6**B shows schematic representation of the multiple sequence alignment of FF proteins. Sequence alignment of conserved sequence motifs is shown. Alignment (color) code was according to the Clustal X color scheme with 40% conservation color increment in Jalview software (16). FIG. **6**C shows schematic representation of secondary structure prediction of AFF-1 based on the multiple sequence alignment of FF proteins. The consensus prediction is shown—alpha helices are marked as tubes (originally represented by red tubes), and beta sheets as arrows (originally represented by green arrows).

FIGS. **7**A-D—FF-1 protein from *P. pacificus* can fuse *C. elegans* cells. The gene Ppa-ff-1 (Table 2) was PCR amplified from *P. pacificus* genomic DNA and cloned downstream to a heat shock promoter (hsp16.2) from *C. elegans*. Transgenic worms were generated using microinjection of wild type stain (N2) and crossed to eff-1 (ok1021) (8). Ectopic cell fusion was visualized by following the disappearance of an apical junction marker from fusing membranes (AJM-1::GFP) using confocal Z series reconstruction (2, 8) (n=9 embryos). FIGS. **7** A-D shows pictograms of ectopic cell fusion of transgenic worms (FIGS. **7**A-B are fluorescence images, FIG. **7**C is bright field and FIG. **7**D is a merger of images of FIGS. **7**B and **7**C). 1.5-fold stage embryos, anterior to the left and ventral down. The effect of ectopic cell fusion is lethal. Scale bars are 10 μm. FIG. **7**A shows a wild type embryo, two dorsal hypodermal cells undergo normal fusion (represented by asterisk). Unfused junction is marked with an arrow. FIG. **7**B-D—eff-1 mutant embryo expressing hsp::Ppa-ff-1 (SEQ ID NO:20) after heat shock. The disappearance of apical junctions between individual cells suggests that Ppa-FF-1 mediates fusion of the hypodermal cells in an eff-1 independent manner.

FIGS. **8**A-B. shows a schematic model of predicted structural homology of AFF-1 to class II viral fusogens. FIG. **8**A—Top: model of Dengue 2 envelope glycoprotein E (accession: GI: 34811077/8). The following domains are shown: (Domain II (representing pink), Domain I (representing gray), Domain III (representing yellow) of the resolved structure of Dengue 2 E protein (PDB:loke)). Bottom (boxed)—Model of AFF-1 putative fusion ("fusion loop") and ij ("jl loop") loops.

FIG. **8**B—(B) Structural alignment of the Dengue 2 E protein and of the predicted secondary structure of AFF-1. Fusion loop and ij loop are indicated by lines above the text. Alignment color is according to the Clustal X coloring scheme. Beta sheets—boxes and black arrows below text; Alpha helixes—boxes and black lines below text. Background: Domain II ((representing Pink), indicated by "II"), Domain I ((representing Gray), indicated by "I"), Domain III ((representing Yellow), indicated by "HI").

DETAILED DESCRIPTION OF THE INVENTION

Definitions

To facilitate an understanding of the present invention, a number of terms and phrases are defined below. It is to be understood that these terms and phrases are for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

The term "construct", as used herein, refers to an artificially assembled or isolated nucleic acid molecule which may include one or more nucleic acid sequences, wherein the nucleic acid sequences may include coding sequences (that is, sequence which encodes an end product), regulatory sequences, non-coding sequences, or any combination thereof. The term construct includes, for example, vector but should not be seen as being limited thereto.

"Expression vector" refers to constructs that have the ability to incorporate and express heterologous nucleic acid fragments (such as, for example, DNA), in a foreign cell. In other words, an expression vector comprises nucleic acid sequences/fragments (such as DNA, mRNA, tRNA, rRNA), capable of being transcribed. Many prokaryotic and eukaryotic expression vectors are known and/or commercially available. Selection of appropriate expression vectors is within the knowledge of those having skill in the art.

The term "expression", as used herein, refers to the production of a desired end-product molecule in a target cell. The end-product molecule may include, for example an RNA molecule; a peptide or a protein; a virus; and the like; or combinations thereof.

As used herein, the terms "introducing" and "transfection" may interchangeably be used and refer to the transfer of molecules, such as, for example, nucleic acids, polynucleotide molecules, vectors, and the dike into a target cell(s), and more specifically into the interior of a membrane-enclosed space of a target cell(s). The molecules can be "introduced" into the target cell(s) by any means known to those of skill in the art, for example as taught by Sambrook et al. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York (2001), the contents of which are incorporated by reference herein. Means of "introducing" molecules into a cell include, for example, but are not limited to: heat shock, calcium phosphate transfection, PEI transfection, electroporation, lipofection, transfection reagent(s), viral-mediated transfer, and the like, or combinations thereof. The transfection of the cell may be performed on any type of cell, of any origin, such as, for example, human cells, animal cells, plant cells, virus, nematode cell, stem cells, cancer cells, and the like. The cells may be selected from isolated cells, tissue, cultured cells, cell lines, cells present within an organism body, and the like.

As referred to herein, the terms "nucleic acid", "nucleic acid molecules" "oligonucleotide", "polynucleotide", and "nucleotide" may interchangeably be used herein. The terms are directed to polymers of deoxyribonucleotides (DNA),

ribonucleotides (RNA), and modified forms thereof in the form of a separate fragment or as a component of a larger construct, linear or branched, single stranded, double stranded, triple stranded, or hybrids thereof. The term also encompasses RNA/DNA hybrids. The polynucleotides may include sense and antisense oligonucleotide or polynucleotide sequences of DNA or RNA. The DNA or RNA molecules may be, for example, but not limited to: complementary DNA (cDNA), genomic DNA, synthesized DNA, recombinant DNA, or a hybrid thereof or an RNA molecule such as, for example, mRNA, tRNA, shRNA, siRNA, miRNA, and the like. The terms further include oligonucleotides composed of naturally occurring bases, sugars, and covalent internucleoside linkages, as well as oligonucleotides having non-naturally occurring portions, which function similarly to respective naturally occurring portions.

The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

The term "homology", "homologous" and "homologs" as used herein are directed to sequence similarity between different sequences of peptides or different sequences of nucleic acids. For example, if two or more proteins have highly similar amino acid sequences, it is likely that they are homologs. In some embodiments, homologs may include homologs within species, between species and/or between phyla. In some embodiments, the term homologs include orthologs and/or prologs.

As referred to herein, the term "exogenous gene" is directed to a gene (or any part thereof) which is introduced from the exterior into a cell. In some embodiments, the exogenous gene is inserted in the form of a polynucleotide (for example, DNA, RNA, and example, in the form of an expression vector. In some embodiments, the exogenous gene is capable of being expressed in the cell. In some embodiments, the exogenous gene is overexpressed within the cell.

As referred to herein, the term "Kill" with respect to a cell/cell population/organism is directed to include any type of manipulation that will lead to the death of that cell/cell population/organism.

The terms "treating" and "treatment" as used herein refer to reduction in severity and/or frequency of symptoms, elimination of symptoms and/or underlying cause, prevention of the occurrence of symptoms and/or their underlying cause, and improvement or remediation of damage. Thus, for example, "treating" a subject involves prevention of a particular disorder, or infection or adverse physiological event in a susceptible subject as well as treatment of a clinically symptomatic subject.

As referred to herein, the terms "anthelmintic(s)" or "antihelminthic(s)" or "anti-nematode" may interchangeably be used. The terms are directed to agents/compositions that are directed against helminths (parasitic round worms or nematodes). The agents/compositions may include various molecules, such as, for example, but not limited to chemical compound, drug, nucleic acid molecule (such as, for example, DNA, RNA, siRNA, ribozyme, modified nucleic acids and the like), a protein or a peptide (such as, for example, an enzyme, an antibody, and the like), a toxin, or combinations thereof. In various embodiments, the antihelminthic may be used to stun, inhibit/suppress growth, and/or kill helminths cells. In some embodiments, the term "antinematodal" relates to antihelminthic which are directed against nematodes. In some embodiments, the terms antihelminthic and antinematodal may interchangeably be used. In some embodiments, the antinematodal is nematocidal (that is an agent that is able to kill the nematode/nematode cell). In some embodiments, the antinematodal is nematostatic (that is an agent that is able to stun/inhibit/suppress growth of the nematode/nematode cell).

As referred to herein, the terms "fusion protein", "fusogen" and "fusogenic protein" may interchangeably be used. The terms are directed to a protein/peptide which is able to induce/mediate cell to cell fusion, for example, through fusion of the lipid bi-layers of the cells. In some embodiments, the fusogenic protein is an endogenous protein (that is, a protein encoded by the authentic genome of the cell and is usually expressed by an unmodified cell). In some embodiments, the fusogenic protein is an exogenous protein (that is, a protein which is encoded by a foreign gene introduced into the cell). In some embodiments, the fusogenic protein comprises a portion/domain of the full length fusogenic protein. The portion of the fusogenic protein may be any domain of the fusogenic portion or combinations of such domains, such as, for example, Signal peptide (SP) domain; Pro-domain; TGFβ-RI-like domain; "[LMF]-G-W-[YFL]-[RK] motif" domain; the Putative protein-protein interaction domain; Membrane proximal stem domain, Trans-membrane domain (TM), Fusion loop, and the like, or any desired peptide derived from the fusogenic protein sequence.

As referred to herein, the term "FF protein(s)" is directed to Fusion Family proteins. The term "CeFF proteins" is directed to FF proteins of *C. elegans* origin. Members of the FF proteins include, for example, AFF-1 and/or EFF-1 proteins and homologs thereof. Wherein said fusogenic protein of nematode origin it is meant to include members of the Fusion Family (FF) proteins as well as homologs thereof, wherein homologs may include homologs within species, between species and/or between phyla. In some embodiments, the term "homologs" include orthologs and/or paralogs. As referred to herein, homologs of the FF Family protein are proteins that share primary and/or secondary amino acid sequence signatures including a pattern of cysteins in the ectodomain of the type I membrane glycoprotein. To consider membership to the FF family the candidate protein is to share at least 15% identity or similarity with a known FF or protein of related secondary or tertiary structure (as further demonstrated in FIGS. **6**A-C). In some embodiments, an FF protein may be selected from any of the FF proteins listed in Table 4 below herein. In some exemplary embodiments, FF proteins may be selected from, but no limited to: CeAFF-1 (SEQ ID NO: 23), CeEFF-1 (SEQ ID NO:24), tsp-FF-1 (SEQ. ID. NO: 25), Bfl-FF-1 (SEQ ID NO: 26), CeEFF-2, Cbr-aff-1, Cbr-eff-1, Cre-aff-1, Cre-eff-1, Cs5-AFF-1, Cs5-EFF-1, Cs7/9-AFF-1, Cs7/9-EFF-1, Cs11-FF, Ppa-FF-1, Ppa-FF-2, Ppa-FF-3, Pen-FF-1, Pma-FF-1, Tsp-ff-2, Tps-FF, Tpa-FF, Min-FF, Mar-FF, Mha-FF, Gpa-FF, Gpa-FFA, Gpa-FFB, Aca-FF, Bma-FF-1, Hco-FF, Asu-FF, Oti-FF, Oti-EFF-1, Dim-FF, Hao-FF, Lsi FF 1, Lsi-FF-2, Hgl-FF, Tmu-FF-1, Tmu-FF-2, Sra-FF-1, Sra-FF-2, Sra-FF-3, Ovo-FF, Tci-FF, Wba-FF, Llo-FF, Bfl-FF-3, Ppi-FF, Cfi-FF, Lsa-FF, Ngr-FF-1, Ngr-FF-2, Ngr-FF-3, Ngr-FF-4, Bxy-FF-1, Bxy-FF-2, Can-FF, Hba-FF-1, Hba-FF-2, Rcu-FF, Hpo-FF, Ana-FF-1, Ana-FF-2, Mle-FF-1A, Mle-FF-1B, Mle-FF-2, MeI-FF-3, MeI-FF-4, MeI-FF-5, MeI-FF-6 or any combination thereof. Each possibility is a separate embodiment.

As used herein, the term "pseudotyped virus" relates to a virus in which the endogenous viral envelope proteins have been replaced by envelope proteins from other sources, such as, for example, from other viruses, by exogenous proteins or peptides (for example, of nematode origin), and the like.

As referred to herein, the term "virus cell" is meant to include a virus, viral particle, viral envelope, viral vector and/or pseudotyped virus.

As used herein, the term "bald virus" relates to an enveloped viral particle or a pseudoviral particle lacking one or more viral envelope proteins.

As used herein, the term "homotypic" with respect to fusion is directed to fusion between cells that express the same fusion protein, or cells that express fusogenic protein of the same family.

As used herein, the term "non-insect cell" is directed to include cells which are not of insect origin. The term includes, for example, such cells as, mammalian cells, avian cells, plant cell, viral particle, human cells, animal cells, and the like.

As referred to herein, where a color is mentioned by name with respect to a figure (for example, "red", "cyan", "purple", "green", "pink", "yellow", etc.) it refers to the color as can be identified in the figure when reproduced in color scale. Where applicable, and as indicated in the brief description of the Figures, where a color is mentioned it is further indicated by an identifier, such as, arrow (solid, dash, long dash arrow head, and the like), asterisk, box, numerical or any other indication. The original figures reproduced in color can be found in publication by some of the inventors of the present application (Avinoam, et. al. (19), the contents of which is incorporated by reference herein in its entirety.

According to some embodiments, and as exemplified herein, nematode fusogenic proteins of the FF family and homologs thereof, such as for example, CeFF proteins, may be capable of mediating fusion of a virus to a cell (such as, for example, a mammalian cell), when the FF proteins are expressed and presented on the membranes of the virus and the cell. In some embodiments, the FF proteins expressed by the virus are replacing the endogenous fusogenic proteins of the virus. In some embodiments, the fusogenic protein expressed by the virus is the same fusogenic protein as that expressed by the cell. In some embodiments, the fusogenic protein expressed by the virus is not the same fusogenic protein as the fusogenic protein expressed by the cell, but rather a family member thereof. For example, the fusogenic protein expressed by both the virus and the cell is AFF-1 protein or homologs thereof. For example, as further shown below, the fusion protein expressed by the virus is AFF-1 whereas the fusion protein expressed by the cell is EFF-1, and vice versa. Accordingly, in some embodiments, a nematode fusogenic protein may replace the endogenous viral fusogen as the minimal fusogenic machinery. Moreover, the infection biology of the virus may be transformed from a mechanism in which the endogenous viral fusogen, (for example, VSVG), is required only in the virus, to a homotypic, fusion protein-dependent mode-of-action in which the fusogen(s) is expressed in the membranes of both the virus and the target cell.

According to some embodiments, the fusion family protein may be any member of the family of fusogenic proteins that may be of nematode origin, or even of other phylum, such as, for example, chordates. Sequence comparisons (ref. 4, and herein) identified putative FF members in thirty five nematode species, suggesting that the FF family is conserved in the phylum Nematoda (4). In addition, similar proteins were found in the arthropods *Calanus finmarchicus* and *Lepeophtheirus salmonis* (Crustacea), the ctenophore *Pkurobrachia pileus*, the chordate *Branchiostoma floridae* (order Amphioxi) and the protist *Naegleria gruberi*, (as shown in FIG. **4**A and Table 4 below), suggesting that FF proteins are conserved in at least four animal phyla and one protist. Phylogenic analysis of full and partial FF sequences revealed that FF proteins may be classified into three subgroups (EFF-1-like, AFF-1-like and FF; FIG. **4**A). To further characterize the molecular conservation of FF proteins, a multiple sequence alignment (FIG. **6**A) was generated and determined that FF proteins share a common organization of putative conserved domains (FIG. **6**B). For example, FF proteins share a pattern of cysteins in the TGFb-RI-like domain (Domain III in FIGS. **6**A-C), suggesting they may be conserved at the level of protein structure. As shown in FIG. **6**C, secondary structure predictions show that they may belong to the family of "mostly beta sheet super family"). Additionally, as further demonstrated hereinbelow, sequence structure analyses and comparisons of Hidden Markov models suggest that FF proteins may fold to resemble class II fusogens from alfa and flavi viruses.

According to some embodiments, and as further demonstrated below, FF proteins are a family of membrane fusogens in nematodes that may be interchangeable between species and even beyond the nematode phylum and hence homologs of the family can be used for fusion of cells, when expressed on the membranes of those cells. As exemplified hereinbelow (for example, in FIG. **4**), various homologs of the CeFF proteins (such as, for example, Tsp-FF-1 of the *Trichinella spiralis* nematode species), EFF-1 homolog from the nematode *Pristionchus pacificu* and chordate *B. floridae* (Bfl-ff-1) of the chordates phylum) are able to fuse cells, when expressed by the cells.

According to some embodiments, Fusion Family proteins of nematode origin and homologs thereof can mediate homotypic or heterotypic fusion of a virus and a cell without additional membrane co-factors. The fusion of the virus and the cell results in infection of the cell by the virus.

According to other embodiments, the Fusion Family proteins of nematode origin and homologs thereof can mediate homotypic fusion of two cells (a first cell and a second cell), wherein the cells express the fusogenic protein of nematode origin. In some embodiments, the cells are not-insect cells. In some embodiments, at least one of the fused cells is not an insect cell. Each possibility is a separate embodiment.

According to some embodiments, the nematode may be any type of nematode. For example, the nematode may be selected from, but not limited to: *Caenorhabditis elegans, Caenorhabditis briggsae, Caenorhabditis japonica, Caenorhabditis ramanei, Caenorhabditis brenneri, Caenorhabditis* sp5,7,9,11, *Trichinella spiralis, Trichinella pseudospiralis, Trichinella papuae, Pristionchus entomophagus, Pristionchus maupasi, Pristionchus pacificus, Meloidogyne incognita, Meloidogyne arenaria, Meloidogyne hapla Globodera pallida, Ancylostoma caninum, Brugia Malayi, Haemonchus contortus, Ascaris suum, Oscheius tipulae, Dirofilaria immitis, Howardula aoronymphium, Litomosoides sigmodontis, Heterodera glycines, Romanomermis culicivorax, Trichuris muris, Strongyloids ratti, Onchocerca volvulus, Teladorsagia circumcincta, Wuchereria bancrofti, Loa loa*, and any other nematode known in the art. Each possibility is a separate embodiment.

According to some embodiments, in order to express an exogenous fusogenic protein in a cell, the cell may be introduced with a construct, such as, for example, an appropriate expression vector, encoding the desired fusion pro-

tein. The construct that encode for the fusogenic protein may include a plasmid, vector, viral construct, or others known in the art, used for replication and expression in the appropriate target cell (which may include, for example, mammalian cells, avian cell, plant cells, viruses, and the like). The construct may be used for transient transfection and/or stable transfection of the cells. Expression of the fusogenic protein can be regulated by any promoter known in the art to act in the target cell. Such promoters can be inducible or constitutive. Such promoters include, for example, but are not limited to: the SV40 early promoter region, the promoter contained in the 3' long terminal repeat of Rous sarcoma virus, the herpes thymidine kinase promoter, the regulatory sequences of the metallothionein gene, the viral CMV promoter, the human chorionic gonadotropin-beta promoter, and the like. Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the construct which can be introduced directly into the cell, by any method known in the art. Alternatively, when the target cell is not a virus, viral vectors can be used which selectively infect the desired target cell. In some embodiments, expression of the fusogenic protein in the cell is transient. In other embodiments, expression of the fusogenic protein in the cell is chronic. In some embodiments, expression of the fusogenic protein in the cell may be inducible (that is, the fusogenic protein is expressed only under certain conditions).

According to some embodiments, there is thus provided a method for a specific, protein-mediated virus to cell fusion, wherein the virus and the cell express a fusogenic protein of the nematode family, or homologs thereof. The method includes placing/incubating/mixing the virus and the cell in close proximity, thereby allowing interaction of the fusogenic proteins which consequently may lead to fusion of the cells. According to some embodiments, the fusogenic protein is selected from AFF-1, EFF-1 and homologs thereof. In some exemplary embodiments, the fusogenic protein is a CeFF protein. In some embodiments, the same fusogenic protein is expressed on the surface of both the virus and the cell. In some embodiments, each of the virus and the cell express a different fusogenic protein, both fusogenic proteins belong to the family of Fusion Family proteins. In some embodiments, for at least one of the cells, the fusogenic protein is an exogenous protein. The method may be performed in-vitro and/or in vivo. When performed in vitro, the virus and the cell may be placed in the same growth medium and incubated under favorable conditions which allow the growth of the cells.

According to some embodiments, there is further provided a method for a targeted and specific fusion of a nematode cell and a virus expressing an exogenous fusogenic protein of nematode origin, wherein the exogenous fusogenic protein expressed by the virus replaces an endogenous fusogenic protein that may be expressed by the virus.

In some embodiments, the virus expressing the exogenous fusogenic protein of nematode origin may be any type of virus capable of fusing with other cells. In some embodiments, the native (unmodified) virus may be capable of inducing fusion by virtue of endogenous fusogenic proteins expressed by the unmodified virus. For example, but without limitation, the virus may be of the retrovirus family (for example, HIV, MLV); Hepadnavirus, Poxyvirus, Rhabdoviridae viruses (for example, Vesicular stomatitis virus (VSV)); Paramyxoviruses; Herpes viruses; Coronavirus, and the like, or combination thereof.

According to further embodiments, there is thus provided a method for the targeted killing or inhibition of a nematode/nematode cell, the method comprises contacting the nematode with a virus expressing an exogenous fusogenic protein of nematode origin, thereby allowing fusion of the virus to a cell of the nematode. Consequent to the fusion between the virus and the nematode cell, the virus may induce lysis of the nematode/nematode cell and/or inhibit the growth of the nematode/nematode cell. The nematode may further become unlivable if too many of its cells are killed.

According to further embodiments, there is provided a method for a specific, protein mediated, cell to cell fusion. The method includes having the cells express a fusogenic protein on their membranes and mixing/placing/incubating the cells in close proximity, thereby allowing interaction of the fusogenic proteins which may lead to fusion of the cells. The protein which mediates the fusion may be a fusogenic protein of nematode origin or homologs thereof, and is expressed on the surface of the cells and thereby allows fusion of the cells. According to some embodiments, the fusogenic protein is selected from AFF-1, EFF-1 and homologs thereof. In some embodiments, the fusogenic protein is a CeFF protein. In some embodiments, the same fusogenic protein is expressed on the surface of both cells. In some embodiments, each cell expresses a different fusion protein, both fusogenic proteins belong to the family of Fusion Family proteins. In some embodiments, the fusogenic proteins are endogenously expressed proteins. In some embodiments, for at least one of the cells, the fusogenic protein is an exogenous protein. In some embodiments, the cells are of similar origin. In some embodiments, the cells are of different origin. For example, the cells may be of human origin, animal origin, plant origin, avian origin, and the like. In some exemplary embodiments, both cells (i.e. the first cell and the second cell) express an exogenous Ce-AFF-1 protein. In some exemplary embodiments, both cells (i.e. the first cell and the second cell) express an exogenous Ce-EFF-1 protein. In some embodiments, the first cell expresses an exogenous Ce-AFF-1 protein and the second cell expresses a Ce-EFF-1 protein. In some embodiments, upon fusion of the cells (i.e. the first cell and the seconds cell), and hybrid cell is formed. In some embodiments, the hybrid cell contains two nucleus.

According to additional embodiments, the method for the specific, protein mediated, cell to cell fusion may be used to specifically target antinematodal agents to nematodes. In such embodiments, fusion of a nematode cell to a foreign cell is dependant on the expression of a fusogenic protein of nematode origin (or homologs thereof) in the membranes of both fusing cells, wherein the fusogenic proteins may be identical or different. In some exemplary embodiments, one cell is of nematode origin (endogenously expressing the fusion protein) and the second cell is of different/foreign origin (such as, for example, of viral origin, plant origin, mammalian origin, avian origin, insect origin), wherein the second cell exogenously expresses a fusogenic protein of nematode origin. The second cell may comprise one or more antinematodal agents that may include any antinematodal agent known in the art or to be developed/identified in the future. The antinematodal agent that is comprised in the cell may be further carried in a carrier within the cell, wherein the carrier is configured to protect the agent within the cell. The carrier may include, for example, such carriers as, liposomes, vacuoles, capsules, microspheres, micelles, and the like. In some embodiments, the antinematodal agent is encoded/produced by the cell. Upon specific fusion of the cells, the antinematodal agent may be released/expressed and exert an effect on the nematode. The effect may be, for example, kill, stun, and/or inhibit/suppress growth of the nematode.

According to some exemplary embodiments, the antinematodal agent may be selected from, but not limited to: a chemical compound (such as, for example, but not limited to: organophospates, carbamates, imidazole derivatives, such as, for example, benzimidazole, Levamisole, Fumigant nematicides, macrolides, avermectin, milbemycin and the like); a nucleic acid (such as, for example, antisense DNA molecules directed against nematode genes; siRNA molecules directed against nematode genes, and the like); proteins (such as, for example, but not limited to: an enzyme capable of cleaving a nematode protein, an antibody directed against a nematode protein); toxins, antibodies and combinations thereof.

According to further embodiments, the method for the specific, protein mediated, cell to cell fusion may thus be used for the treatment of parasitic nematode infection of various organisms (such as, for example, animals and humans) and plants. In some embodiments, the method may include inducing one or more cells of the organism/plant to be treated to express a fusogenic protein of a nematode origin and an antinematodal agent, whereby upon expression of the fusogenic protein by the cell, a nematode infecting the organism or plant is fused to said cell expressing the fusion protein, thereby exposing the nematode to the antinematodal agent.

In some embodiments, a transgenic plant is provided, in which at least some of the plant cells have been modified to express a fusogenic protein of the nematode family and optionally further express an antinematodal agent (such as, for example a protein or a peptide having antinematodal effect, nucleic acid sequence capable of exerting an antinematodal effect, and the like). Upon infection of the transgenic plant with a nematode, the nematode cells will fuse with the plant cells expressing the fusogenic proteins, and the antinematodal agent comprised/encoded by those cells may exert a deleterious effect on the nematode, such as, for example, kill the nematode and/or inhibit/suppress its growth. In some embodiments, the expression of the nematode fusogenic protein by plant cells is constitutive (that is, the cells constitutively express the nematode fusion protein). In some embodiments, the expression of the nematode fusogenic proteins by plant cells are induced under different conditions (such as, for example, different lighting conditions, different watering conditions, different temperatures, different humidity, and the like, or combinations thereof).

According to some embodiments, the stable or transient expression of a nematode fusogenic protein in plants may be achieved by stable or transient transfection of plant cells with a nucleic acid encoding a nematode fusogenic protein. In stable transformation, the nucleic acid molecule encoding a nematode fusogenic protein is integrated into the plant genome, and as such it represents a stable and inherited trait. In transient transformation, the nucleic acid molecule is expressed by the cell transformed but not integrated into the genome, and as such represents a transient trait.

There are various methods of introducing foreign genes into both monocotyledonous and dicotyledonous plants. The principal methods of the stable integration of exogenous DNA into plant genomic DNA include two main approaches: (i) *Agrobacterium*-mediated gene transfer, which includes the use of plasmid vectors that contain defined DNA segments which integrate into the plant genomic DNA. Methods of inoculation of the plant tissue vary depending upon the plant species and the *Agrobacterium* delivery system. A widely used approach is the leaf-disc procedure, which can be performed with any tissue explant that provides a good source for initiation of whole-plant differentiation. A supplementary approach employs the *Agrobacterium* delivery system in combination with vacuum infiltration. The *Agrobacterium* system is especially useful for in the creation of transgenic dicotyledenous plants. (ii) Direct DNA uptake. There are various methods of direct DNA transfer into plant cells. In electroporation, the protoplasts are briefly exposed to a strong electric field, opening up mini-pores to allow DNA to enter. In microinjection, the DNA is mechanically injected directly into the cells using micropipettes. In microparticle bombardment, the DNA is adsorbed on microprojectiles such as magnesium sulfate crystals or tungsten particles, and the microprojectiles are physically accelerated into cells or plant tissues.

Following stable transformation, plant propagation then occurs. The most common method of plant propagation is by seed. Another method of regenerating a transformed plant is by micropropagation, which provides a rapid, consistent reproduction of the transformed plants.

Transient transformation of, for instance, leaf cells, meristematic cells, or the whole plant may also be used.

Transient transformation can be effected by any of the direct DNA transfer methods described above or by viral infection using modified plant viruses.

Viruses that useful for the transformation of plant hosts include, for example, cauliflower mosaic virus (CaMV), tobacco mosaic virus (TMV), and baculovirus (BV). In some embodiments, pseudotyped BD virus expressing FF proteins can also be used to deliver toxins, nucleic acids and other molecules.

In addition, the nucleic acid molecule encoding a nematode fusogenic protein can also be introduced into a chloroplast genome.

According to additional embodiments, treatment of parasitic nematode infection of organisms such as mammals, avians, rodents, and the like, may include providing the infected organism with a composition (such as, for example, a pharmaceutical composition), which includes a virus particle/viral vector, which is genetically engineered to express an exogenous fusogenic protein of the nematode family. In some embodiments, the virus does not express an endogenous fusogenic protein. In exemplary embodiments, the viral endogenous protein is replaced by a fusogenic protein of the nematode origin. The virus cell/viral vector of the composition does not affect any of the cells of the organism, and upon specific fusion with a nematode cell, can induce killing or inhibition of growth of the nematode. The composition may be formulated by any method known in the art, such as disclosed, for example in the latest edition of "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., which is herein fully incorporated by reference. The composition may further include one or more excipients, as known in the art. The composition may be administered by any administration route, such as, for example, oral, rectal, transmucosal, especially trans-nasal, intestinal, or parenteral delivery, including intramuscular, subcutaneous, and intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. In some embodiments, the composition is formulated for veterinary use. In some embodiments, the composition is in form of a viral vector, wherein administration of viral vectors can be performed by, for example, intravenous or subcutaneous injection into the organism.

Following injection, the viral vectors can circulate until they recognize nematode cells, whereby they viral vector fuses to the nematode cells and induce their killing or suppression of their growth.

In some embodiments, the virus cell/viral vector, which is genetically engineered to express an exogenous fusogenic protein of the nematode family may be integrated into the genome of the treated organism.

In some embodiments, a transgenic animal is provided, in which at least some of the cells have been modified to express a fusogenic protein of the nematode family and optionally further express an antinematodal agent (such as, for example a protein or a peptide having antinematodal effect, nucleic acid sequence capable of exerting an antinematodal effect, and the like).

According to some embodiments, there is provided a method for treating a nematode infection in a subject, comprising administering to the subject a composition comprising a cell expressing an exogenous fusogenic protein of the nematode family, wherein fusion of said cell of the composition and a nematode cell infecting the subject, may lead to, the death and/or inhibition of growth of the nematode, thereby treating the nematode infection. In some embodiments, the subject is human. In some embodiments, the composition is a pharmaceutical composition that may be formulated by any method known in the art. In exemplary embodiments, the composition is formulated to be administered orally and to release the cell expressing the nematode fusogenic protein in the intestines.

In additional embodiments, there is provided a use of a composition comprising a cell expressing an exogenous nematode fusogenic protein and optionally an antinematodal agent, for the treatment of nematode infection in a subject in need.

According to some embodiments, there is provided a method for treating nematode infection in an animal, comprising administering to the animal a composition comprising a cell expressing an exogenous fusogenic protein of the nematode family, wherein fusion of said cell of the composition and a nematode cell may lead to the death and or inhibition of growth of the nematode, thereby treating the nematode infection in the animal. In some embodiments, the animal is a rodent, a mammal, an avian, and the like. In some exemplary embodiments, the animal is cattle, chicken, horse, canine, and the like, or any other animal that may be infected by nematode.

According to some embodiments, there is further provided a viral vector for the expression of a nematode fusogenic protein on the surface of a virus. The fusogenic protein may be selected from AFF-1, EFF-1 and homologs thereof.

According to further embodiments, there is provided a cell expressing an exogenous fusogenic protein of nematode origin, wherein said exogenous fusogenic protein is a Fusion Family protein member. The cell may be of any origin, such as, for example mammalian cell, avian cell, viral cell, plant cell, human cell, animal cell, and the like. In some embodiments, the cell is a non-insect cell. Each possibility is a separate embodiment.

In various embodiments, there are further provided kits for practicing antinematodal methods of various embodiments. The kits may include, for example, at least one or more of a virus expressing an exogenous nematode fusogenic protein; a cell expressing an exogenous nematode fusion protein, wherein the cell may optionally express an antinematodal agent; a vector for expressing a nematode fusogenic protein on the surface of a cell; and a viral vector expressing a nematode fusogenic protein. The kits may further include additional components, such as, for example, suitable containers, suitable growth medium, buffers, reagents, and the like. Additionally, the kit may further include instructions for using the components of the kit for practicing various embodiments, such as, for example, for the treatment of nematode infection.

According to various embodiments, it is to be emphasized that wherein said fusogenic protein of nematode origin, it also encompasses homologs thereof. In some embodiments, the fusogenic protein comprises any protein having at list 15% identity or similarity with a known FF or protein of related structure (as demonstrated, for, example, in FIG. **6**).

The term comprising includes the term consisting of.

While a number of exemplary aspects and embodiments have been discussed above, those of skill in the art will recognize certain modifications, permutations, additions and sub-combinations thereof. It is therefore intended that the following appended claims and claims hereafter introduced be interpreted to include all such modifications, permutations, additions and sub-combinations as are within their true spirit and scope.

The following examples are presented in order to more fully illustrate certain embodiments of the invention. They should in no way, however, be construed as limiting the broad scope of the invention. One skilled in the art can readily devise many variations and modifications of the principles disclosed herein without departing from the scope of the invention.

EXAMPLES

Materials and Methods

DNA Constructs

For transient expression of FF proteins, AFF-1::FLAG (SEQ ID NO:1), EFF-1::V5 (SEQ ID NO: 2), Tsp-FF-1::FLAG (SEQ ID NO: 3), and Bfl-FF-1::FLAG (SEQ ID NO: 4) were inserted into the pCAGGS mammalian expression vector (15) (Tables 2 and 4). Unless otherwise indicated, 5' KpnI and 3' NheI restrictions sites were used to clone into pCAGGS. To generate pOA20 (Table 2) the DNA encoded by pIZT-AFF-1 (10) was PCR amplified using primers OR55, OR56 (Table 3). To generate pOA19 (Table 2) the DNA encoded by pIZT-EFF-1A (9) was PCR amplified using primers OR54, OR55 (Table 3). To generate pOA35 (Table 2), DNA from a cDNA library (obtained from Nagano) was PCR amplified using nested primers OR100-OR103 (Table 3). The PCR product was ligated into pGEMT-easy as recommended by manufacturer (Promega) and then used as template for PCR amplification with primers OR111 and OR112 (Table 3). To generate pOA60 (Table 2), the cDNA sequence corresponding to accession gi|210090015| with Flanking 5' KpnI 3' NheI was optimized for expression and synthesized (GeneScript). To label cytoplasm, pRFPnes (16) was used. To label the nucleus, pCFPnls (SEQ ID NO. 21) encoding CFP with two tandem repeats of the nuclear localization signal (nls) from simian virus large T-antigen was used. To generate pCFPnls, primers OR147-148 (Table 3) were used with pCH44 (16) as template. The PCR product was cloned into the BamHI, EcoRI sites of pcDNA3.1 (+) (Invitrogen). To generate pOA6 *P. pacificus* genomic DNA (PS312) was used as template with primers OR-19 and OR-22 (Table 3). The PCR product was ligated into pPD49.78. To generate pRSETA-AFF1EC (SEQ. ID. NO: 27), primers AM66 and AM 67 (Table 3) were used with Ce-AFF-1 cDNA as template (10). The PCR product was cloned into the BglII, KpnI sites of pRSET-A (Invitrogen). All sequences were verified by sequencing.

TABLE 2

Plasmids used

Plasmid Name	Description
pOA6	*P. pacificus* eff-1 genomic sequence (SEQ ID NO: 20) in pPD49.78 (hsp 16.2)
pOA19	*C. elegans* eff-1 fused to a C-terminal V5 tag (EFF-1::V5) (SEQ ID NO 2) in pCAGGS vector. (V5 tag nt. 1974-2049)
pOA20	*C. elegans* aff-1 fused to a C-terminal FLAG tag (AFF-1::FLAG) (SEQ ID NO: 1) in pCAGGS vector
pOA35	*T. spiralis* ff-1 with a kozak sequence fused to a C-terminal FLAG tag (Tsp-FF-1::FLAG) (SEQ ID NO: 3) in pCAGGS vector (FLAG-tag nt. 1779-1803 of SEQ ID NO: 3).
pOA60	*B. floridae* ff-1 with a kozak sequence fused to a C-terminal FLAG tag (Bfl-FF-1::FLAG) (SEQ ID NO: 4) in pCAGGS vector (FLAG tag: nt. 1740-1764 of SEQ ID NO: 4)
pCFPnls	CFP with two tandem nuclear localization signals (SEQ ID NO: 21)
pCAGGS	A chicken beta-actin/rabbit beta-globin hybrid promoter with a human *cytomegalovirus* immediate early promoter (CMV-IE) enhancer
pCAGGS-Gind	VSV G Indiana strain (ref. 20)
pRFPnes	DsRed2 with a nuclear export signal (pCH19) (Ref. 16)
pRSETA-AFF1EC including 6His tags	The extracellular domain of Ce-AFF-1 including 6-His tag (SEQ ID NO. 27). (6XHis tags, nt. 1692-1710 of SEQ ID NO: 27).

TABLE 3

Primers used

Name	Sequence
OR-19 (SEQ ID NO: 5)	ATGATCTTCTCTTCTCTTCTACTGTATAC
OR-22 (SEQ ID NO: 6)	TCATACATAATCTCCAGGTAGAACATC
OR-54 (SEQ ID NO: 7)	TTAATTGGTACCACTATGGAACCGCCGTTTGAGTGG
OR-55 (SEQ ID NO: 8)	AATTAAGCTAGCTCAACCGGTACGCGTAGAATCGAGACC
OR-56 (SEQ ID NO: 9)	TTAATTGGTACCACTATGGTACTGTGGCAATGGTCAATAGCC
OR-100 (SEQ ID NO: 10)	ATGTTCTCACCACTTTTTTGTCTTCTTCTTCTGC
OR-101 (SEQ ID NO: 11)	AACTGCCTCGCCCAAGAATATGCC
OR-102 (SEQ ID NO: 12)	ATATTCTTGGGCGAGGCAGTTGACC
OR-103 (SEQ ID NO: 13)	TCACAATTTGTTAGCATTCGTTCTGCC
OR-111 (SEQ ID NO: 14)	TTAATTGGTACCATGTTCTCACCACTTTTTTGTCTTCTTCTTCTGC

TABLE 3-continued

Primers used

Name	Sequence
OR-112 (SEQ ID NO: 15)	AATTAAGCTAGCTCATTTGTCATCGTCGTCCTTGTAGTCCAATTTGTTAGCATTCGTTCTGCCATTTCC
OR-147F (SEQ ID NO: 16)	AATTAAGGATCCATGGTGAGCAAGGGCGAGGAGCTG
OR-148R (SEQ ID NO: 17)	AATTAAGAATTCTTATACCTTTCTCTTCTTTTTTGGATCTACC
AM-66 (SEQ ID NO: 18)	TATGTCTTAGATCTCCAAAGTCTCATCAGTACACAGTACT
AM-67 (SEQ ID NO: 19)	TGTATCATGGTACCCTCTGTGAAATCCCCACCATGAGC

Nematode Strains

Nematode strains were maintained according to standard protocols. In addition to the wild-type strain N2, the following strains were used LGII: BP347 eff-1 (ok1021) (9). LGIV: SU93 jcIs1[ajm-1::gfp, pRF4] (7), BP421 eff-1 (ok1021)II; hyEx161[ajm-1::gfp, (21) pOA6 (Ce-hsp::Ppa-ff-1) (SEQ ID NO 20). To drive Ppa-ff-1 ectopic expression in *C. elegans,* 10 ng/μl of pOA6 (Table 2) were co-injected with 10 ng/μl of the apical junction marker AJM-1: GFP (hyEx161).

Bioinformatics

Identification and Characterization of New Members of the FF Family

FF proteins in nematodes were identified as described in Reference 4. For the Chordate, Ctenophore and Arthropod sequences, the BLAST search provided by the National Center for Biotechnology Information (NCBI) was used. For annotation, the Augustus gene prediction software with the training set for *C. elegans* was used. In some cases, the gene model was manually corrected based on the multiple sequence alignment (for example, as shown in FIG. **6**B). Accession numbers and databases are summarized in Table 4, hereinbelow.

Phylogeny of FF proteins (FIG. **4**A)

Phylogenetic analyses were conducted in MEGA4. The evolutionary history was inferred using the Maximum Parsimony (MP) method. The MP tree was obtained using the Close-Neighbor-Interchange algorithm with search level 3 in which the initial trees were obtained with the random addition of sequences (10 replicates). All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option). The signal sequence was removed from the final dataset. Evolutionary relationships of 14 (right) and 25 (left) taxa is shown (FIG. **4**A). For 14 taxa, the full length sequence of the extracellular domain was used Tree #1 out of 3 most parsimonious trees (length=1165) is shown. The consistency index is (0.926554), the retention index is (0.930667), and the composite index is 0.868356 (0.862313) for all sites and parsimony-informative sites (in parentheses). There were a total of 438 positions in the final dataset, out of which 344 were parsimony informative. For 25 taxa the amino acid sequences corresponding to the TGFβ-RI like domains (10) AFF-1 (84-192) (Residues 84-192 of SEQ ID NO: 23 were

used; the sequence of the *N. gruberi* served as an outgroup. Tree #1 out of 9 most parsimonious trees (length=469) is shown. The consistency index is (0.727876), the retention index is (0.717241), and the composite index is 0.529138 (0.522063) for all sites and parsimony-informative sites (in parentheses). There were a total of 75 positions in the final dataset, out of which 60 were parsimony informative.

Secondary Structure Prediction (FIG. **6**C)

Predictions were performed using the JNET method available from the web services of the Jalview 2.5 software.

Structural Homology (FIGS. **8**A-B)

The multiple sequence alignment was used as query to scan the protein data bank (PDB) for homologues by sequence-structure comparison either using FUGUE v2.s.07 or HHpred. Searches identified several putative distant homologues belonging to the Class II family of viral fusogens (14). The most probable homology was to Dengue2 envelope glycoprotein (loke, lok8, (accession: GI: 34811077/8)) (FUGUE confidence 95% and HHpred probability 61%). In addition, Tick-Born Encephalitis envelope glycoprotein (lsvb), Semliki Forest Virus (lala), and Dengue 1 and 3 were identified with lower probabilities (lp58, luzg).

Cells and Reagents

All Baby Hamster Kidney cells (BHK) are BHK-21 (ATCC). BHK cells and their growth conditions were according to standard protocols. Dulbecco's modified Eagle's medium (DMEM), Penn/Strep, L-glutamine, and sodium pyruvate obtained from Gibco. Fetal Bovine Serum was obtained from Biological Industries, Kibbutz Beit Haemek, Israel. Experiments with Sf9 cells and their growth conditions were as described (9,10).

Cell-Cell Fusion Assay

BHK cells at ~70% confluence were transfected using Fugene6 (Roche) with 2 μg of pCAGGS DNA (including an insert (FF coding sequence as detailed above) or an empty vector) and 0.5 μg of pRFPnes DNA in 35 mm tissue culture dishes (Corning) containing a glass cover slip on the bottom (Knittel). At 14-24 hours post transfection the cells were fixed with 4% paraformaldehyde in PBS and processed for immunofluorescence. To assay multinucleated cells, cell nuclei was stained with Hoechst (1 μg/ml, H3570, Molecular Probes) or 1 μg/ml DAPI for 10 min at room temperature (9). The number of nuclei in expressing cells as marked by pRFPnes or antibody staining (see below), was counted using either a Zeiss Axiovert 200M inverted or a Nikon Eclipse E800 upright fluorescence microscope. The fusion indexes (shown as percentage of fusion) were defined as the ratio between the number of nuclei in multinucleated cells and the total number of nuclei in fused cells and expressing cells that were in contact but did not fuse. The fusion indexes are presented as means±standard errors of at least eight independent experiments. Each experiment consisted of at least two replicates of the same transfection (2, 3). Transfection efficiency was evaluated as 40-60% based on pRFPnes and antibody staining.

Color Mixing Assay

Cytoplasmic content mixing assays were performed as described (16) with some modifications. The cytoplasm of cells that express AFF-1 was marked with a red fluorescent protein by expressing RFPnes. The nuclei of cells that express EFF-1 were marked with a CFPnls. Fused hybrid cells could be distinguished by their red cytoplasm surrounding multiple blue nuclei. The percentages of fused hybrid cells (red and cyan; purple) and multinucleated cells (red or cyan alone) were calculated by dividing the mean number of red, cyan and purple cells by the mean number of cells from four independent experiments. Experiments were repeated at least five times yielding similar results independent of whether the co-transfection fluorescent marker was RFPnes or CFPnls.

Pseudoviruses Preparation

Recombinant viruses were recovered as described (17) with some modifications. BHK cells were grown to 70% confluence on 10 cm plates and then transfected with plasmids encoding pCAGGS empty vector, pOA19 or pOA20 (Table 3). Following 24 hour incubation at 37° C. in 5% CO_2, cells were infected with VSVG-complemented VSVΔG recombinant virus (VSVΔG-G) at a multiplicity of infection (MOI) of 2-5 for 1 hour at 37° C. in a 5% CO_2 incubator in serum free culture medium (DMEM). Virus infected cells were washed at least 3 times with serum-free DMEM or PBS to remove unabsorbed VSVΔG-G virus. Following a 24 hour incubation period at 37° C. the supernatant Wand cells containing the VSVΔG, VSVΔG-EFF-1, or VSVΔG-AFF-1 pseudoviruses were harvested and centrifuged at 600 g for 10 min at 4° C. to clear cell debris. Virions were removed from the supernatant by pelleting at 100,000 g through a 20% sucrose cushion and resuspended in 10% sucrose in Hepes/NaCl buffer (25 mM Hepes, 130 mM NaCl pH 7.4).

Titering VSV Pseudotype Viruses on BHK Cells

To determine the titer of each pseudovirus preparation, 3×10^4 BHK cells were plated into each well of a 96 well tissue culture plate (NUNC). For titering of VSVΔG-AFF-1 or VSVΔG-EFF-1, BHK cells were initially transfected with 1 μg/ml aff-1 or eff-1, pOA20 or pOA19, respectively. Cells transfected with empty vector served as control. Six serial dilutions of the virus were performed and added to cells. After 18-24 hours of incubation, GFP expressing cells were counted in at least two dilutions using a Zeiss Axiovert 200M fluorescence microscope. Each experiment was repeated at least three times with duplicates. Inoculation was performed in the presence of anti-VSVG antibody mAb I1 diluted 1:100 to inhibit infection due to residual, presence of VSVG. Results were also confirmed by FACS analysis. For FACS analysis BHK cells were grown to 70% confluence and transfected with 1 μg/ml of plasmid encoding aff-1 or eff-1 (plasmids pOA20 or pOA19, respectively). Following 24 hour incubation, cells were infected with VSVΔG-AFF-1 and incubated for 24 hours. To measure the titer cells were collected using EDTA and fixed in 4% paraformaldehyde. Samples were maintained on ice and examined for GFP expression using BD FACS Calibur (N=20,000 cells, FIG. **5**).

Immunoblotting

To detect proteins by Western blotting, samples were treated with SDS-PAGE sample buffer containing 10% of β-mercaptoethanol or RIPA buffer (50 mM Tris, 150 mM NaCl, 1% NP40, 5% Deoxycholate) for 20 min at 4° C. Samples were boiled in the presence of 20 mM DTT for 5 min and the protein profile was examined on an 8%, 10% or 12% SDS polyacrylamide gel. For AFF-1 expressing cells (BHK-AFF-1) and viruses (VSVΔG-AFF-1) bands were visualized using mouse anti-FLAG (M2, Sigma F3165) monoclonal antibody and mouse anti-M polyclonal antibody (FIG. **1**B). For EFF-1 expressing cells (BHK-EFF-1) and viruses (VSVΔG-EFF-1) bands were visualized using mouse anti-V5 (Cat #46-0705 Invitrogen). In controls, rabbit anti-VSVG (Cat #V4888 Sigma-Aldrich) was used. As secondary antibodies goat anti-mouse antibodies conjugated with HRP (Cat #115-035-003 Jackson), were used. Bands were detected by chemoluminescence (EZ-ECL kit, Biological Industries, Kibbutz Beit Haemek, Israel) using a FUJI

LAS 3000 with the Image Gauge V3.12 software package. Data shown are representative of at least three independent experiments.

Production of Mouse Anti-AFF-1 Polyclonal Antibodies

The extracellular domain of AFF-1 (AFF-1EC) was subcloned into pRSET-A that introduced 6×His at the N terminus (Table 2). The AFF-1EC::6×His (SEQ ID NO: 27) fusogenic protein was over-expressed in *E. coli* by adding 0.5 mM IPTG and incubating the culture overnight at 16° C. Rosetta and affinity purification with NiNTA beads (Qiagen Cat#30210) was according to the QIAexpressionist manual (06/2003, QIAGEN). The protein was eluted by adding four 0.5 ml aliquots of elution buffer A (8M urea, 100 mM NaH_2PO_4, 10 mM Tris-HCl, pH 5.9) followed by another four aliquots of elution buffer B (8M urea, 100 mM NaH_2PO_4, 10 mM Tris-HCl, pH 4.5). Mouse polyclonal antibodies were prepared using AFF-1EC as antigen (Adar Biotech Inc., Israel).

Immunofluorescence

BHK-21 cells were grown on tissue culture plates with glass cover slips on the bottom (Knittel). Cells were fixed with 4% paraformaldehyde in PBS, incubated in 40 mM NH_4Cl to block free aldehydes, washed in PBS, permeabilized in 0.1% triton X-100 in PBS and blocked in 1% FBS in PBS. The cover slips were incubated 1 hour at 23° C. with either anti-V5 1:500 (Invitrogen) or anti-FLAG 1:2000 (Sigma) mouse monoclonal antibodies. The secondary antibodies were goat anti-mouse and goat anti-rabbit coupled to Alexa488, 633 or 643 (Molecular Probes/Invitrogen). Transfected cells expressed cytoplasmic RFP from the pRFPnes vector and nuclei were visualized with DAPI or Hoechst staining as described above.

Sere obtained from mice immunized with AFF-1EC were tested by immunofluorescence using Sf9 cells expressing AFF-1::Flag. Sera #8 diluted 1:500 showed membrane and intracellular vesicular staining (FIG. **2**N, Panel A). Preimmune sera (FIG. **2**N, Panel B) or secondary antibodies alone (Alexa Fluor 568 goat anti-mouse IgG (H+L) 1:500) gave no staining.

Transmission Electron Microscopy (TEM)

Negative Staining-TEM

A 400-mesh carbon-coated grid was placed on a 20 μl sample drop for 2 min and blotted with a filter paper. The sample was chemically stained by placing the grid on a 20 μl drop of 2% uranyl acetate for 2 min followed by blotting with a filter paper and air-drying. Specimens were examined in a Tecnai T12 G^2 TEM (FEI) or in a Philips CM120 transmission electron microscopes operated at 120 kV. Images were recorded digitally on a Gatan UltraScan 1000 2k×2k camera or the Gatan 791 wide-angle camera using the DigitalMicrograph software (Gatan, U.K.)

Cryo Electron Microscopy

A 3-μl drop of the sample was placed on a glow-discharged holey carbon coated copper electron microscopy grid (C-flat, Protochips). The drop was blotted, and the sample was vitrified by plunging into liquid ethane (−183° C.). The specimen was then transferred to liquid nitrogen (−196° C.) for storage. Vitrified specimens were examined on a Tecnai F30 Polara TEM (FEI) operated at 300 kV and equipped with a GIF2002 postcolumn energy filter (Gatan) operated in zero loss mode. 2K×2K images were acquired at a calibrated magnification of 27,500×, resulting in a pixel size of 0.5 nm at the specimen level. Projection images were recorded at defocus settings between −4 μm to −6 μm using SerialEM. Alternatively, specimens were examined in a Tecnai T12 G^2 TEM (FEI) operated at 120 kV and images were recorded digitally on a Gatan UltraScan 1000 2K×2K camera using the DigitalMicrograph software (Gatan, U.K.).

Cryo Electron Tomography

A 4-μl aliquot of the pseudotyped virus preparations was pipetted onto a glow-discharged holey carbon coated copper electron microscopy grid (Cflat, Protochips). Colloidal 10 nm diameter gold particles coupled to bovine serum albumin (BSA) were added, excess liquid was absorbed using a filter paper and the grids were vitrified by plunge-freezing in liquid ethane. Vitrified grids were stored in liquid, nitrogen until examined on a Tecnai Polara TEM (FEI) operated at 300 kV and equipped with a GIF2002 or Tridem postcolumn energy filter (Gatan) operated in zero loss mode. 2K×2K images were acquired at a calibrated magnification of 27,500×, resulting in a pixel size of 0.5 nm at the specimen level. Tilt series were collected at a defocus of either −6 μm for the viral tomogram or −8 μm for the vesicle tomogram in two-degree increments covering an angular range from −60° to 60° using SerialEM. The total electron dose was kept below 100 electrons/$Å^2$. Tilt series were aligned using gold beads as fiducials. Three dimensional reconstructions were calculated from the tilt series in IMOD using weighted back projection. Slices for figures were prepared using Amira 5.2 (Visage Imaging).

Measurements

The width and length of the particles on the surface of viruses and vesicles were measured from images of negatively stained samples using ImageJ Software 1.410. The G glycoprotein was measured as control and the obtained size was compared to published dimensions. Unpaired t tests were performed ($P<0.0001$).

Immunogold Labeling

Virus samples were pipetted onto carbon-coated grids and incubated for 5 min and then blocked with 1% BSA in PBS for 30 min at room temperature. The grids were then placed on a 50 μl drop of anti-AFF-1 (#8 serum; see Immunofluorescence section above) diluted 1:100 in PBS containing 1% BSA and incubated overnight at 4° C. in a sealed humidified chamber. Excess antibody was removed by placing grids sequentially onto three 50 μl drops of 0.1% BSA in PBS for 2 min each time. The grids were then placed on a 20 μl drop of goat anti-mouse IgG conjugated with 12-nm gold particles (Jackson lab, 1:20) for 1 hour at room temperature. Unbound gold conjugates were removed by three sequential 2-min washes with PBS. Samples were fixed by placing the grids on a 50 μl drop of 0.1% glutaraldehyde in PBS for 5 min. The grids were washed twice in PBS for 2 min and were then negatively stained by incubating the grids for 2 min on a 20 μl drop of 2% phosphotungstic acid in water (pH 7). Excess stain was removed and the grids were air-dried. Images were recorded digitally as described above.

Example 1

CeFF Proteins are Capable of Mediating Virus-Cell Fusion

In order to test whether CeFF proteins are capable of mediating virus-cell fusion when presented on the membrane of Vesicular Stomatitis Virus (VSV), AFF-1 complemented VSVΔG pseudoviruses, in which the gene encoding the fusogenic glycoprotein (VSVG) was replaced by GFP (VSVΔG-AFF-1; scheme of FIG. **1**A) were generated. Infection of Baby Hamster Kidney cells (BHK) expressing AFF-1 (BHK-AFF-1) on their surface with VSVΔG-AFF-1 showed a 600-fold increase in infection compared to control BHK cells (FIG. **1**C to **1**E). These results demonstrate that

AFF-1 can replace the endogenous viral fusogen as the minimal fusogenic machinery that can mediate virus-cell binding and fusion. To explore whether EFF-1 and AFF-1 can heterotypically interact with each other, VSVΔG-AFF-1 was added to BHK expressing EFF-1 (BHK-EFF-1, Scheme of FIG. **1**C) and vice versa. It was found that VSVΔG-AFF-1 was able to infect BHK-EFF-1 cells and that the efficiency of AFF-1-AFF-1 and AFF-1-EFF-1 mediated virus-cell fusion was not significantly different (FIG. **1**D). Although infection due to residual VSVG complemented VSVΔG (VSVΔG-G) was negligible (FIG. **1**D), inoculations in the presence of neutralizing anti-G antibody mAb I1 were performed to assure that only AFF-1-mediated infection was measured (FIG. **1**F). The results further demonstrate that AFF-1 and EFF-1 can mediate homotypic virus-cell fusion without additional membrane co-factors and AFF 1-EFF-1 mediated fusion also resulted in infection.

Example 2

Structure-Function of AFF-1

To study the relationship between structure and function of AFF-1, transmission electron microscopy (TEM) was used. Negatively stained samples of VSVΔG to VSVG and AFF-1 complemented VSVΔG preparations were compared. VSVΔG virions have the typical VSV 'bullet' shape with a smooth membrane, hence termed bald, while both VSVΔG-G and VSVΔG-AFF-1 virions displayed distinct spikes on their envelopes (FIG. **2**A to C). In negative stain (pH 5), VSVG form elongated spikes on VSVΔG-G (FIG. **2**B), while VSVΔG-AFF-1 show bulkier spikes (FIG. **2**C). The estimated average spike length of VSVG and AFF-1 as measured from the negative stain images were 145 Å and 110 Å respectively (Table 1). To confirm that the observed spikes were indeed AFF-1, immunogold labeling using anti-AFF-1 polyclonal antibodies was performed. A specific immunoreactivity on the surface of VSVΔG-AFF-1 is observed (FIGS. **2**D, **2**E, **2**N and **2**O). To further characterize the pseudoviruses at higher resolution and in a more native state they were imaged embedded in vitreous ice by cryo electron microscopy (cryoEM, FIG. **2**F to H) and cryo electron tomography (cryoET, FIG. **2**I to K). CryoEM projection images show that AFF-1 proteins uniformly coat the pseudoviruses. Individual spikes could be observed at central sections of the tomograms (FIG. **2**J, inset). Higher order assemblies of AFF-1 in the form of penta- or hexamerit "flower" shaped complexes could be observed in computational slices through the tomogram oriented peripheral to the pseudotyped virus particles (FIG. **2**I, inset). These assemblies were even better visible in slices through the tomograms of co-purified vesicles (FIGS. **2**L, **2**M and **2**P). The order of these arrays may have a critical function in bending and deforming plasma membranes to mediate fusion.

TABLE 1

Measured size of AFF-1 and VSVG

	VSVG		AFF-1	
	Length (nm)	Width (nm)	Length (nm)	Width (nm)
Mean Size	14.5	8.7	10.9	6.0
SEM	0.5	0.4	0.5	0.3

TABLE 1-continued

Measured size of AFF-1 and VSVG

	VSVG		AFF-1	
	Length (nm)	Width (nm)	Length (nm)	Width (nm)
N	20		32	
Size (38)	12.5	6.0		

*SEM—Standard Error, N—number of measurements

Example 3

FF Proteins can Interact

To show that FF proteins can interact, cytoplasmic mixing between cells using a color mixing-assay (FIG. **3** A to C) was performed. aff-1 was coexpressed with a fluorescent protein (red) that contained a nuclear export signal (RFPnes; FIG. **3**A) and the cells were mixed with cells co-expressing eff-1 and a fluorescent protein (cyan) that contained a nuclear localization signal (CFPnls; FIG. **3**B). The two cell populations were co-cultured and multinucleated cells were observed, mostly dikaryons, expressing both markers (FIGS. **3** C, E to H and O). In contrast, no cells expressing both markers following mixing of cells transfected with empty vector (FIGS. **3** D and L) were observed. AFF-1-mediated mixing (FIG. **3**I to K) occurred only when the protein was expressed in both cells (FIGS. **3** M and N); thus, cytoplasmic mixing during cell-cell fusion is dependent on the expression of AFF-1 in both fusing partners.

Example 4

Divergent FFs can Function as Fusogens

To determine whether divergent FFs can function as fusogens, Tsp-ff-1 was expressed in BHK cells and its activity was compared to AFF-1 (FIGS. **4** B and D). Using immunofluorescence 28±4% multinucleation was observed in cells transfected with Tsp-ff-1 compared to 26±2% and 4±3% multinucleation in cells that were transfected with aff-1 and empty vector, respectively (FIG. **4**F). In addition, EFF-1 paralog from the nematode *Pristionchus pacificus* was expressed in *C. elegans* embryos to result in ectopic fusion of embryonic cells (FIG. **7**). Additionally, expression of the FF ortholog identified in the chordate *B. floridae* (Bfl-ff-1), (FIGS. **6**A and **6**B and Table 4), in BHK cells resulted in 37±7% multinucleation (FIGS. **4** E and F).

Example 5

Identification and Characterization of New Members of the FF Family

FF proteins in nematodes were identified as described in (4). For the Chordate, Ctenophore and Arthropod sequences the BLAST search provided by the National Center for Biotechnology Information (NCBI) was used. For annotation, the Augustus gene prediction software with the training set for *C. elegans* was used. In some cases, the gene model was manually corrected based on the multiple sequence alignment (FIG. **6**B). Accession numbers and databases are summarized in Table 4, hereinbelow.

TABLE 4

Sequence identifiers/Accession numbers

Species	Sequence Identifier/Accession number
Caenorhabditis elegans	CeAFF-1 (SEQ ID NO: 23) (WP: CE41369) CeEFF-1 (SEQ ID NO: 24) (WP: CE03028, WP: CE30881, WP: CE31159, WP: CE32594) C26D10.7 (CeEFF-2) (WP: CE36985)
Caenorhabditis briggsae	Cbr-aff-1 (CBP17138) Cbr-eff-1 (BP: CBP34546, BP: CBP37650)
Caenorhabditis japonica	CJA05978 (CJA05978/JA11265) CJA03218 (CJA03218/JA03456)
Caenorhabditis ramanei	Cre-aff-1 (RP: RP19336) Cre-eff-1 (RP: RP32670, RP: RP36929)
Caenorhabditis brenneri	Database: Caenorhabditis_ (CBN32067, CN35418, Database: Caenorhabditis_PB2801-4.0-contigs [Contig1645.2]) CBN17896 (CBN17896, CN30500, CN18501)
Caenorhabditis sp5, 7, 9, 11	Cs5-AFF-1(Database: Caeno_sp5_DRD-2008_JU800_2.fna, contig2451) Cs5-EFF-1(Database: Caeno_sp5_DRD-2008_JU800_2.fna, contig6234) Database: Caeno_sp5_DRD-2008_JU800_1.fna [contig_262626] Database: Caeno_sp7_ju1286_454scaffolds_1.fna [scaffold00005] Database: Caeno_sp9_ju1422_454scaffolds_1.fna [scaffold00235, scaffold00002] Cs7/9-AFF-1 (Database: Caeno_sp7_ju1286_454scaffolds_1.fna scaffold00007; Database: Caeno_sp9_ju1422_454scaffolds_1.fna scaffold00001, scaffold00169) Cs7/9-EFF-1 (Database: Caeno_sp7_ju1286_454scaffolds_1.fna scaffold00005; Database: Caeno_sp9_ju1422_454scaffolds_1.fna scaffold00002) Cs7/9-EFF-1(Database: Caeno_sp9_ju1422_454scaffolds_1.fna scaffold00235) Cs7/9-EFF-1(Database: Caeno_sp9_ju1422_454scaffolds_1.fna scaffold00235) Cs11-FF (gi|319532004|gb|AEKS01003493.1|, Contig629.1546) Database: Caeno_sp11_JU1373_454scaffolds_1.fna [scaffold01488]
Pristionchus pacificus	Ppa-FF-1 (Database: PpaFreeze1.bases Contig235.2) Ppa-FF-2 (Database: PpaFreeze1.bases Contig162.2) Ppa- FF-3 (Database: PpaFreeze1.bases Contig735.1 + Contig735.2)
Pristionchus entomophagus	Pen-FF-1 (Database: Pristionchus_entomophagus-3.0.bases Contig1225.3)
Pristionchus maupasi	Pma-FF-1 (Database: p. maup genome Contig3990.1)
Trichinella spiralis	gi|162730680 (Tsp-ff-1) (gi|339236477|ref|XP_003379793.1) gi|339234943|ref|XP_003379026.1| gi|339234945|ref|XP_003379027.1|
Trichinella pseudospiralis	Tps-FF gi|149208398|gb|EF601568.1| (SEQ ID NO.
Trichinella papuae	Tpa-FF gi|149208399|gb|EF601569.1|
Meloidogyne incognita	Min-FF (gi|198718377|emb|CABB01003673.1| *Meloidogyne incognita*, whole genome shotgun sequence assembly, contig_3673, gi|19265127|gb|BM881383.1|BM881383 rb09d06.y1), gi|19265127|
Meloidogyne arenaria	Mar-FF (BI746953, rm34a12.y1) gi|15768755|
Meloidogyne hapla	Mha-FF (gi|207096946|gb|ABLG01001006.1| *Meloidogyne hapla* strain VW9 Mh10g200708_Contig1005, gi|207095745|gb|ABLG01002207.1| *Meloidogyne hapla* strain VW9 Mh10g200708_Contig2206)
Globodera pallida	Gpa-FF (gi|54548408|gb|CV578685.1|CV578685 kf14e11.y1) Gpa-FFA (Database: gpal.201011.contigs.fasta [Sanger] Contig 1004319) Gpa-FFB (Database: gpal.201011.contigs.fasta [Sanger] Contig 1004319)
Ancylostoma caninum	Aca-FF (gi|157997724|gb|EX544342.1|EX544342 AIAC-aaa88e02.g1) Aca-FF (gi|158000776|gb|EX547394.1|EX547394 AIAC-aaa14c06.g1) gi|157990577|
Brugia Malayi	Bma-FF-1 (gi|170576008|ref|XP_001893468.1| hypothetical protein Bm1_09975) gi|170582744|ref|XP_001896266.1| hypothetical protein Bm1_24045 gi|170576006|ref|XP_001893467.1| hypothetical protein Bm1_09970 gi|154234139|gb|AAQA01001677.1| *Brugia malayi* ctg_62087 gi|154209490|gb|AAQA01025369.1| *Brugia malayi* ctg_35248 gi|154234539|gb|AAQA01001277.1| *Brugia malayi* ctg_54442 Bm1_09970 + Bm1_09975 Bm1_24045
Haemonchus contortus	Hco-FF (gi|27320801|gb|CA869252.1|CA869252 px01a04.y1) Hco-FF (Database: HAEM.contigs.fasta [Sanger] Contig 006057)
Ascaris suum	Asu-FF (gi|113050648|gb|ED245151.1|ED245151 AUAC-aag39g05.g1, gi|320312531|gb|AEUI01008540.1| *Ascaris suum* ASU_contig008540) gi|320301910|gb|AEUI01019161.1| *Ascaris suum* ASU_contig019161 gi|320309474|gb|AEUI01011597.1| *Ascaris suum* ASU_contig011597 gi|320297040|gb|AEUI01024031.1| *Ascaris suum* ASU_contig024031
Oscheius tipulae	Oti-FF (Database: Oscheius_tipulae_clc3_1.fna, contig 5292) Oti-EFF-1 (Database: Oscheius_tipulae_clc3_1.fna, contig 4684) Database: Oscheius_tipulae_clc3_1.fna [Contig 4684, contig 5292]
Dirofilaria immitis	Dim-FF (Database: Dirofilaria_immitis_v1.3_Maker_Transcripts.fna, DimmContig4043_DIMM48125; Database:

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Species	Sequence identifiers/Accession numbers Sequence Identifier/Accession number
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Howardula aoronymphium	Hao-FF (Database: Howardula_aoronymphium_clc_1.fna, contig_147926) Hao-FF (Database: Howardula_aoronymphium_clc_1.fna, contig_103402) Database: Howardula_aoronymphium_clc_1.fna
Litomosoides sigmodontis	Lsi-FF-1 (Database: Litomosoides_sigmodontis_abyss_1.fna, 342875) Lsi-FF-2 (Database: Litomosoides_sigmodontis_abyss_1.fna, 344431, 332390) Database: Litomosoides_sigmodontis_abyss_1.fna
Heterodera glycines	Hgl-FF (gi|170569983|gb|ABLA01000927.1|)
Romanomermis culicivorax	contig05859, contig06497 (Kindly provided by W. Kelley Thomas)
Trichuris muris	Tmu-FF-1 (Database: T_muris_contigs.fasta [Sanger]; NODE_192365_length_12375_cov_11.496000) Tmu-FF-2 (Database: T_muris_contigs.fasta [Sanger]; NODE_99371_length_23257_cov_11.624845)
Strongyloids ratti	Sra-FF-1 (Database: RATTI.contigs.fasta [Sanger] Contig 75311) Sra-FF-2 (Database: RATTI.contigs.fasta [Sanger] Contig 74980) Sra-FF-3 (Database: RATTI.contigs.fasta [Sanger] Gontig 75430) Database: S.ratti.reads
Onchocerca volvulus	Ovo-FF (Database: O_volvulus_all454_contigs.fna [Sanger], contig25914)
Teladorsagia circumcincta	Tci-FF (Database: T_circumcincta_reads.fasta [Sanger] Supercontig_0000831)
Wuchereria bancrofti	Wba-FF (gi|285840565|gb|ADBV01004176.1| cont1.4176) GI: 285822425, GI: 285835743
Loa loa	Llo-FF (gi|285859024|gb|ADBU01000052.1| contig1.52) GI: 285852521, GI: 285851695
Branchiostoma floridae	Bfi-FF-1 (Database: fgenesh2_pg.scaffold_465000022; Protein ID: 104514)jgi|Brafl1|104514|fgenesh2_pg.scaffold_465000022 (SEQ ID NO. 26) Bfl-FF-3 (Database: fgenesh2_pg.scaffold_46500002; Protein ID: 104513) jgi|Brafl1|104513|fgenesh2_pg.scaffold_465000021
Pleurobrachia pileus	Ppi-FF (gi|167791107|gb|CU417832.1|CU417832)
Calanus finmarchicus	Cfi-FF (gi|190134016|gb|FG632618.1)
Lepeophtheirus salmonis	Lsa-FF (gi|293020530|gb|ADND01294772.1|) gi|293020530|
Naegleria gruberi	Ngr-FF-1 (gi|284087402|gb|EFC41072.1|) Ngr-FF-2 (gi|284087338|gb|EFC41008.1|) Ngr-FF-3 (gi|284083966|gb|EFC37664.1| NAEGRDRAFT_81886) Ngr-FF-4 (gi|284083965|gb|EFC37663.1| NAEGRDRAFT_81885) est's GI: 168534442; GI: 168542950
Bursaphelenchus xylophilus	Bxy-FF-1 (gi|351002770|emb|CADV01009240.1) Bxy-FF-2 (gi|351002016|emb|CADV01009994.1|
Caenorhabditis angaria	Can-FF (gi|308940912|gb|AEHI01101512.1|, contigRNAPATHr2484_10)
Heterorhabditis bacteriophora	Hba-FF-1 (gi|343491313|gb|ACKM01000079.1|, Contig56.1) Hba-FF-2 (gi|343491313|gb|ACKM01000079.1|, Contig56.1)
Romanomermis culicivorax	Rcu-FF
Heligmosomoides polygyrus	Hpo-FF (Database: Heligmosomoides_polygyrus_clc_1.fna, contig_126399) Hpo-FF (Database: Heligmosomoides_polygyrus_clc_1.fna, contig_372600) Hpo-FF (Database: Heligmosomoides_polygyrus_clc_1.fna, contig_376900)
Acrobeloides nanus	Ana-FF-1 Ana-FF-2
Mnemiopsis leidyi	Mle-FF-1A (gi|346671607|gb|AGCP01008927.1| c605800073.Contig1) Mle-FF-1B (gi|346671607|gb|AGCP01008927.1| c605800073.Contig1) Mle-FF-2 (gi|346671607|gb|AGCP01008927.1| c605800073.Contig1) Mel-FF-3 (gi|346680498|gb|AGCP01000036.1| c600000000.Contig54) Mel-FF-4 (gi|346673076|gb|AGCP01007458.1| c605700043.Contig1) Mel-FF-5 (gi|346661390|gb|AGCP01019144.1| c601500011.Contig6) Mel-FF-6 (gi|346674975|gb|AGCP01005559.1| c606400022.Contig1)

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide

<400> SEQUENCE: 4

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aagaacaggt actccttcta caggcccaag tacgagtcca agtgcatctg cgactgcccc 360

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cagtacggcg actactgcaa ctccaagacc aacaggtgta ccgagcacga gctggacttc 420

tgctacaaca cctacaggtc cgaccagacc gcccacggct gcctggccta ctggaactcc 480

gaggagtccg aggtgtgctg cgccctgtac gtgggcaagc accccgagtc ccccaagtac 540

gacgccgtgt acctgtcctc cgacggcaag cccatcgtga agctggccct gaaggtgtac 600

gactccagga ccgacgaggt ggtgtactcc taccccacct tcaccgtggc cctgaacgac 660

aggacccaca gggcccagca ctccgtgagg atggaggtga ccggcgacac ccccacccag 720

atcgactccg gctactacta cgccacctcc gagggcaccc agctgtacac cgacgtgtcc 780

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ggcaccgtgg agaggccccc cgagaggacc gtgctggacg ctttccacct gaagacccac 900

aagtgccaca acaagttcga ctacaccgag acctggcaaa tctacggcga gggcgactgg 960

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cagggctggc tggagctgtt cgccatgctg ctggacatcg ccctgttcat cgccggcatc 1620

ttcctgttca tcaaggtctg cacctgcttc aacgtgttca ccaccaaggc ccccaagtgg 1680

gacgagggcg tggagatgtc cgtgctgagg aggaggaagg ccgagcccgg cgacgtctgc 1740

gactacaagg acgacgacga caagtaa 1767

<210> SEQ ID NO 5
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 5

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<210> SEQ ID NO 6
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 6

tcatacataa tctccaggta gaacatc 27

<210> SEQ ID NO 7
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 7

ttaattggta ccactatgga accgccgttt gagtgg 36

<210> SEQ ID NO 8
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 8

aattaagcta gctcaaccgg tacgcgtaga atcgagacc 39

<210> SEQ ID NO 9
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 9

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<210> SEQ ID NO 10
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 10

atgttctcac cacttttttg tcttcttctt ctgc 34

<210> SEQ ID NO 11
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 11

aactgcctcg cccaagaata tgcc 24

<210> SEQ ID NO 12
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 12

atattcttgg gcgaggcagt tgacc 25

<210> SEQ ID NO 13
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 13

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<210> SEQ ID NO 14
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 14

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<210> SEQ ID NO 15
<211> LENGTH: 69
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 15

aattaagcta gctcatttgt catcgtcgtc cttgtagtcc aatttgttag cattcgttct 60

gccatttcc 69

<210> SEQ ID NO 16
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 16

aattaaggat ccatggtgag caagggcgag gagctg 36

<210> SEQ ID NO 17
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 17

aattaagaat tcttatacct ttctcttctt ttttggatct acc 43

<210> SEQ ID NO 18
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 18

tatgtcttag atctccaaag tctcatcagt acacagtact 40

<210> SEQ ID NO 19
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 19

tgtatcatgg taccctctgt gaaatcccca ccatgagc 38

<210> SEQ ID NO 20
<211> LENGTH: 3584
<212> TYPE: DNA
<213> ORGANISM: Pristionchus pacificus

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<400> SEQUENCE: 20

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cctcgttcat tcactcctca attcaaagta attcctcctc attgtatcaa gactccttcc 120

atcattgctc atccttccaa aggtattctc tatctttact ctcagatgtt agacagaatg 180

agatagaagg ggaataagag agacgcgtag gaagatgaag aggttcttca cactcttgtg 240

caatatcttc ggatatctca gctgatgaaa ctaactagag gatatttcac aagtgataag 300

atcaagtcta aaagaggaaa gatgtcatca atagttctcg tttctcacat tactcatctc 360

tctcattcct cattcctctc atcaactatc ctatcaatta cagtagttca aacatgggca 420

gagtaatggg aatggggtca ttcataactg attcaggaga aggatcactc aaatctctcg 480

agatgcattt ctctattgga ttgcatacaa ctgtctgttt ccgattagag gatacaaatg 540

ataattcgac tcaatccaat caatcattac ttcatacagt tactctatct agtatagaac 600

atcatcatcc agtgagtatt ctacccaatc tccccttctc tgtcttcctc tttatttttc 660

atcttctcag gtcacacaga aatacaactt cggtattcct gaagtaagtg cagattgttt 720

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gatttaattc aaaattaatt aatgtatgaa gaataagaag ggacacacac gaatgatgtt 3060

cacattatga taaattgata atttagattg acgtggagaa taattcgtgg agggaattaa 3120

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cactcaaatt atttggagga ttccaatcac ttggtaatta ttaactagct tcttagtcta 3240

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agtattcatt gaaaaatgtg ttctttcagt gttgtgtatg tcctcttctc gttgccaaaa 3480

taactcaatc tcaacgaaat cgatcacaaa cggaagttga aattgaagaa cgaaggcgaa 3540

aaatgaagag gaataatgat gttctacctg gagattatgt atga 3584

<210> SEQ ID NO 21
<211> LENGTH: 3584
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide

<400> SEQUENCE: 21

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agatagaagg ggaataagag agacgcgtag gaagatgaag aggttcttca cactcttgtg 240

caatatcttc ggatatctca gctgatgaaa ctaactagag gatatttcac aagtgataag 300

atcaagtcta aaagaggaaa gatgtcatca atagttctcg tttctcacat tactcatctc 360

tctcattcct cattcctctc atcaactatc ctatcaatta cagtagttca aacatgggca 420

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agatgcattt ctctattgga ttgcatacaa ctgtctgttt ccgattagag gatacaaatg 540

ataattcgac tcaatccaat caatcattac ttcatacagt tactctatct agtatagaac 600

atcatcatcc agtgagtatt ctacccaatc tccccttctc tgtcttcctc tttatttttc 660

atcttctcag gtcacacaga aatacaactt cggtattcct gaagtaagtg cagattgttt 720

ctgtgaatgt gaatcaacag agagatgttc ttcttcctct tatgaattca ttaaatgtcc 780

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gcggcattct atgatggatc aatgagacaa gtcatcgtat ctcattctga aggaactaat 2040

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cacattatga taaattgata atttagattg acgtggagaa taattcgtgg agggaattaa 3120

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agtattcatt gaaaaatgtg ttctttcagt gttgtgtatg tcctcttctc gttgccaaaa 3480

taactcaatc tcaacgaaat cgatcacaaa cggaagttga aattgaagaa cgaaggcgaa 3540

aaatgaagag gaataatgat gttctacctg gagattatgt atga 3584

<210> SEQ ID NO 22
<211> LENGTH: 4741
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide

<400> SEQUENCE: 22

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ccaacgaccc ccgcccattg acgtcaataa tgacgtatgt tcccatagta acgccaatag 180

ggactttcca ttgacgtcaa tgggtggact atttacggta aactgcccac ttggcagtac 240

atcaagtgta tcatatgcca agtacgcccc ctattgacgt caatgacggt aaatggcccg 300

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tccttttatg gcgaggcggc ggcggcggcg gccctataaa aagcgaagcg cgcggcgggc 660

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ccttattcca tagaaaagcc ttgacttgag gttagatttt ttttatattt tgttttgtgt 2100

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tcctctcctg actactccca gtcatagctg tccctcttct cttatgaaga tccctcgacc 2220

tgcagcccaa gcttggcgta atcatggtca tagctgtttc ctgtgtgaaa ttgttatccg 2280

ctcacaattc cacacaacat acgagccgga agcataaagt gtaaagcctg gggtgcctaa 2340

tgagtgagct aactcacatt aattgcgttg cgctcactgc ccgctttcca gtcgggaaac 2400

ctgtcgtgcc agcggatccg catctcaatt agtcagcaac catagtcccg cccctaactc 2460

cgcccatccc gcccctaact ccgcccagtt ccgcccattc tccgccccat ggctgactaa 2520

ttttttttat ttatgcagag gcgaggccgc ctcggcctct gagctattcc agaagtagtg 2580

aggaggcttt tttggaggct aggcttttgc aaaaagctaa cttgtttatt gcagcttata 2640

atggttacaa ataaagcaat agcatcacaa atttcacaaa taaagcattt ttttcactgc 2700

attctagttg tggtttgtcc aaactcatca atgtatctta tcatgtctgg atccgctgca 2760

ttaatgaatc gccaacgcgc ggggagaggc ggtttgcgta ttgggcgctc ttccgcttcc 2820

tcgctcactg actcgctgcg ctcggtcgtt cggctgcggc gagcggtatc agctcactca 2880

aaggcggtaa tacggttatc cacagaatca ggggataacg caggaaagaa catgtgagca 2940

aaaggccagc aaaaggccag gaaccgtaaa aaggccgcgt tgctggcgtt tttccatagg 3000

ctccgccccc ctgacgagca tcacaaaaat cgacgctcaa gtcagaggtg gcgaaacccg 3060

acaggactat aaagatacca ggcgtttccc cctggaagct ccctcgtgcg ctctcctgtt 3120

ccgaccctgc cgcttaccgg atacctgtcc gcctttctcc cttcgggaag cgtggcgctt 3180

tctcaatgct cacgctgtag gtatctcagt tcggtgtagg tcgttcgctc caagctgggc 3240

tgtgtgcacg aacccccccgt tcagcccgac cgctgcgcct tatccggtaa ctatcgtctt 3300

gagtccaacc cggtaagaca cgacttatcg ccactggcag cagccactgg taacaggatt 3360

agcagagcga ggtatgtagg cggtgctaca gagttcttga agtggtggcc taactacggc 3420

tacactagaa ggacagtatt tggtatctgc gctctgctga agccagttac cttcggaaaa 3480

agagttggta gctcttgatc cggcaaacaa accaccgctg gtagcggtgg ttttttttgtt 3540

tgcaagcagc agattacgcg cagaaaaaaa ggatctcaag aagatccttt gatcttttct 3600

acggggtctg acgctcagtg gaacgaaaac tcacgttaag ggattttggt catgagatta 3660

tcaaaaagga tcttcaccta gatcctttta aattaaaaat gaagttttaa atcaatctaa 3720

agtatatatg agtaaacttg gtctgacagt taccaatgct taatcagtga ggcacctatc 3780

tcagcgatct gtctatttcg ttcatccata gttgcctgac tccccgtcgt gtagataact 3840

acgatacggg agggcttacc atctggcccc agtgctgcaa tgataccgcg agacccacgc 3900

tcaccggctc cagatttatc agcaataaac cagccagccg gaagggccga gcgcagaagt 3960

ggtcctgcaa ctttatccgc ctccatccag tctattaatt gttgccggga agctagagta 4020

-continued

agtagttcgc cagttaatag tttgcgcaac gttgttgcca ttgctacagg catcgtggtg 4080

tcacgctcgt cgtttggtat ggcttcattc agctccggtt cccaacgatc aaggcgagtt 4140

acatgatccc ccatgttgtg caaaaaagcg gttagctcct tcggtcctcc gatcgttgtc 4200

agaagtaagt tggccgcagt gttatcactc atggttatgg cagcactgca taattctctt 4260

actgtcatgc catccgtaag atgcttttct gtgactggtg agtactcaac caagtcattc 4320

tgagaatagt gtatgcggcg accgagttgc tcttgcccgg cgtcaatacg ggataatacc 4380

gcgccacata gcagaacttt aaaagtgctc atcattggaa aacgttcttc ggggcgaaaa 4440

ctctcaagga tcttaccgct gttgagatcc agttcgatgt aacccactcg tgcacccaac 4500

tgatcttcag catcttttac tttcaccagc gtttctgggt gagcaaaaac aggaaggcaa 4560

aatgccgcaa aaaagggaat aagggcgaca cggaaatgtt gaatactcat actcttcctt 4620

tttcaatatt attgaagcat ttatcagggt tattgtctca tgagcggata catatttgaa 4680

tgtatttaga aaaataaaca aatagggttt ccgcgcacat ttccccgaaa agtgccacct 4740

g 4741

<210> SEQ ID NO 23
<211> LENGTH: 589
<212> TYPE: PRT
<213> ORGANISM: Caenorhabditis elegans

<400> SEQUENCE: 23

Met Arg Leu Trp Gln Trp Ser Ile Ala Val Ala Ile Cys Leu Val Met
1 5 10 15

Val Thr Glu Ala Arg Leu Arg Arg His His Arg Lys Arg Arg Phe Val
20 25 30

Ser Ser Asn Phe Asp Glu Phe Tyr Cys Gly Glu Ser Ala His Ala Gln
35 40 45

Ser Gln Phe Glu Glu Glu Arg Glu Ser Asn Ser Ser Lys Val Ser Ser
50 55 60

Val His Ser Thr Gln Phe Asn Trp Gly Leu Asp Asn Thr Ile Cys Ile
65 70 75 80

Lys Leu Gln Asn Val Val His Val Leu Lys Tyr Glu Arg Leu Glu Gln
85 90 95

Arg Tyr Pro Ile Glu Asn Ser Tyr Thr Phe Ser Val Pro Leu Ile Asp
100 105 110

Thr Asn Cys Lys Cys His Cys Tyr Gly Phe Gly Thr Asn Asp Val Cys
115 120 125

Asn Val Glu Lys Tyr Ala Asp Asp Arg Asn Cys Thr Thr Ser Ser Glu
130 135 140

Phe Pro Thr Cys Tyr Thr Lys Tyr His Pro Ala Val Glu Pro Leu Asp
145 150 155 160

Cys Pro Val Thr Ser Ile Pro Ala Lys Ala Cys Cys Asp Ile Lys Leu
165 170 175

Lys Pro Arg Asp Gly Arg Met Phe Arg Ala Val Lys Leu Gln Gln Pro
180 185 190

Ile Asn Asp Met Ile Ile Ser His Ser Ile Phe Ala Asn Asn Ser Gly
195 200 205

Lys Met Met Lys Val Leu Gly Pro Asp Glu Phe Arg Ile Asn Leu Leu
210 215 220

Lys Gly Lys Glu Gln Phe Glu Leu Thr Glu Tyr His Arg Ile Ser Val
225 230 235 240

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Gln Leu Val Ala Ser Ser Pro Gln Gln Gln Leu Arg Glu Gly Met Tyr
                245                 250                 255

Tyr Phe Pro Glu Glu Asn His Asn Asp Leu Arg Glu Gly Lys Ile Asn
            260                 265                 270

Glu Ile Thr Glu Ser Asp Leu Asp Lys Leu Gly Trp Tyr Arg Arg Val
        275                 280                 285

Gly Asn Asp Trp Gln Val Ala Thr Ser Gly Leu Leu Leu Arg Asn Ala
    290                 295                 300

His Lys Val Val Ile Lys Asn Cys Lys Gly Gln Val His Met Asp Gln
305                 310                 315                 320

Phe Ser Gly Thr Lys Asn Phe Val Leu Arg Gly Thr Gln Tyr Asn Asp
                325                 330                 335

Thr Tyr Asn Glu Arg Arg Val Ser Asp Asn Asn Phe Val Arg Ser Val
            340                 345                 350

Lys Val Asp Glu Ser Ser Arg Glu Ile Thr Ile Val His Glu His Gly
        355                 360                 365

Thr Ala Ala Gln Val Ser Leu Lys Thr Asp Thr Arg Pro Asn Leu Thr
    370                 375                 380

Lys Ser Gln Ser Leu Leu Ala Asn Phe Thr Gly Ser Ile Thr Leu Asp
385                 390                 395                 400

His Asp Gly Asn Arg Met Leu Asn Val Thr Phe Phe Gly Val Lys Gly
                405                 410                 415

Thr Val His Ile Lys Met Tyr Val Asn Asp Arg Lys Leu Ile Ala Thr
            420                 425                 430

Phe Ala Cys Thr Ala Gln Phe Gly Thr Ser Leu Lys Asp Asp Gly Ser
        435                 440                 445

Arg Ile Ser Leu Pro Ser Thr Ile Asn Gln Ala Gln Trp Val Cys Ile
    450                 455                 460

Leu Pro Asp Glu Gln Pro Thr Lys Ser Glu Ile Cys Lys Trp Ile Pro
465                 470                 475                 480

Tyr Glu Glu Lys Ala Met Arg Thr Pro Arg Gln Glu Gln Ser Trp Ser
                485                 490                 495

Lys Gly His Ser Pro Cys Ser Gln Ala Glu Cys Asn Ser Leu Lys Ser
            500                 505                 510

Gly Val Ser Asp Leu Phe Pro Trp Ile Met Asn Phe Asp Tyr Phe Met
        515                 520                 525

Ala His Gly Gly Asp Phe Thr Glu Trp Leu Lys Ile Gly Ile His Ile
    530                 535                 540

Val Ile Ala Val Gly Leu Leu Leu Leu Leu Ile Leu Leu Phe Thr Lys
545                 550                 555                 560

Cys Leu Val Pro Leu Ala Cys Cys Ser Leu Ser Ile Pro Phe Lys Asn
                565                 570                 575

Arg Asn Lys Lys Lys Lys Lys Lys Asn Ser Ser Asp Tyr
            580                 585


<210> SEQ ID NO 24
<211> LENGTH: 658
<212> TYPE: PRT
<213> ORGANISM: Caenorhabditis elegans

<400> SEQUENCE: 24

Met Glu Pro Pro Phe Glu Trp Ser Pro Gln Phe Ile Leu Leu Leu Leu
1               5                   10                  15

Ala Val Thr Thr Tyr Gly Phe Pro Leu Glu Glu Lys Phe Asp Gly Leu
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           20                  25                  30

Phe Arg Ala Glu Pro Pro His Cys Ser Lys Thr Pro Ile Val Arg Ala
        35                  40                  45

Gln Thr Ser Gln Asn Ala Met Ser Ser Ile Ala Arg Gly Met Gln Met
    50                  55                  60

Gln Phe Ser Ile Gly Leu His Thr Ala Val Cys Phe Arg Leu Tyr Glu
65                  70                  75                  80

Asp Thr Gln Leu Ala Ser Gln Glu Ile Asn Asp Asp Glu Asn Ala Gly
                85                  90                  95

Asn Gln Thr Ser Leu Leu His Thr Ile Arg Leu Glu Lys Leu Glu His
            100                 105                 110

His His Pro Ile Thr Gln Arg Tyr Thr Phe Gly Ile Pro Glu Val His
        115                 120                 125

Ala Ser Cys Ile Cys Glu Cys Asp Ala Thr Ser Ser Thr Cys Thr Ala
    130                 135                 140

Glu Ser His Gln Phe Thr Ala Cys Pro Glu Ser Asp Lys Ser Asp Glu
145                 150                 155                 160

Thr Ser Ser Cys Tyr Arg Thr Phe Phe Pro Asn Gln Thr Pro Ile Gly
                165                 170                 175

Cys Ser Glu Asp Asp Ile Pro Lys Leu Cys Cys Asp Val Arg Phe Lys
            180                 185                 190

Pro Tyr Lys Asn Met Thr Phe Leu Ala Val Lys Leu Glu Gln Pro Thr
        195                 200                 205

Thr Tyr Ala Thr Phe Val Tyr Ala Ala Tyr Asp Phe Val Asn Gly Tyr
    210                 215                 220

Trp Val Glu Lys Asp Lys Thr Lys Ile Arg Ser Gln Leu Asp Gly Gly
225                 230                 235                 240

Thr Gln Asp Arg His Leu Asp Gln Lys Arg Arg Ile Ser Leu Ala Val
                245                 250                 255

Thr Ala Gly Gly Arg Ala Ser His Gln Leu Glu Thr Gly Met Tyr Phe
            260                 265                 270

Ser Arg Thr Ser Asn Gly Gly Glu Thr Glu Glu Leu Arg Met Gln Pro
        275                 280                 285

Leu Asn Glu Ile Thr Asp Asn Asn Phe Asp Arg Leu Gly Trp Tyr Arg
    290                 295                 300

Met Asp Asp Ser Gly His Phe His Val Asn Asn Gly Val Val Lys Met
305                 310                 315                 320

Asp Asp Ile His Lys Ala Lys Val Lys Asn Cys Lys Glu Gln Thr Tyr
                325                 330                 335

Lys Ser Ile Leu Ser Ala Asn His Tyr Met Pro Gly His Phe Asn Leu
            340                 345                 350

Thr Arg Pro Leu Glu Val Ile Lys Pro Trp Ile Gln Ser Ala Arg Ile
        355                 360                 365

Phe Asp Ser Ser Leu Arg Gln Ala Val Val Thr His Ala Glu Gly Thr
    370                 375                 380

Asn Leu Gln Ile Ser Ile His Leu Asp Asp Glu Val Glu Ser Gln Asn
385                 390                 395                 400

Leu Val Phe Phe His Asn Ala Ser Arg Ile Arg Asp Phe Ser Gly Ser
                405                 410                 415

Ile Ile Val Asp Ser Lys Ser Asn Arg Leu Phe Asn Leu Thr Val Tyr
            420                 425                 430

Glu Ala Ser Gly Lys Ile Asp Gly Ser Val Lys Met Ser Thr Gly Phe
        435                 440                 445
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Gly Ser Asp Thr Ile His Thr Phe Thr Ala Tyr Val Ser Asp Leu His
    450                 455                 460

Ala Ser Asn Arg Ser Met Ile Ile Pro Leu Pro Ala Ile Val Gly Gln
465                 470                 475                 480

Gly Ala Arg Ala Ile Cys Leu Arg Ala Asp Ser Met Ala Asp Ile Asp
                485                 490                 495

Lys Ile Cys His Val Ile Glu Tyr Phe Glu Ser Pro Leu Glu Ile Asp
            500                 505                 510

Leu Val Glu Gly Lys Trp His Glu Met Ile Gly Thr Cys Pro Thr Cys
        515                 520                 525

Asn Gln Ile Asn Phe Asn Gly Met Met Lys Phe Leu Asn Pro Ala His
    530                 535                 540

Trp Ile Lys Gly Ile Ser Ser Ile Gly Asp Gly Val Met Ile Ala Thr
545                 550                 555                 560

Asp Ile Val Val Tyr Leu Gly Val Leu Cys Ile Leu Tyr Leu Leu Ile
                565                 570                 575

Thr Lys Ile Ile Val Pro Leu Val Arg Cys Trp Val Cys Pro Met Ser
            580                 585                 590

Ile Phe Cys Asn Gly Ser Ser Ser Ser Ser Lys Asn Lys Asn Asp Lys
        595                 600                 605

Arg Arg Lys Glu Arg Glu Glu Arg Arg Arg Lys Asp Lys Phe Val Ser
    610                 615                 620

Glu Ser Glu Asp Gly Ala Arg Ser Ser Ser Glu Pro His Asp Thr Leu
625                 630                 635                 640

Ala Arg Tyr His Gly Asn His Ser Glu Arg His Tyr Ser Ser Ser Gln
                645                 650                 655

Tyr Ile


<210> SEQ ID NO 25
<211> LENGTH: 567
<212> TYPE: PRT
<213> ORGANISM: Trichinella spiralis

<400> SEQUENCE: 25

Met Phe Ser Pro Leu Phe Cys Leu Leu Leu Leu Leu Ser Tyr Cys Val
1               5                   10                  15

Met Arg Ser Val Asn Leu Asp Thr Thr Glu Met Thr Met Thr Thr Thr
            20                  25                  30

Met Gln Thr Gln Ile Gly Leu His Glu Thr Gly Cys Phe Phe Val Asn
        35                  40                  45

Leu His Pro Asp Asn Arg Leu Ser Leu Asn Asp Thr Val Glu Glu Ser
    50                  55                  60

Ile Leu Thr Ser Asn Thr Ser Leu Leu His Thr Leu Arg Tyr Glu Ser
65                  70                  75                  80

Thr His Gln Leu Tyr Pro Val Arg Gln Gln Tyr Ile Phe Ala Ile Pro
                85                  90                  95

Glu Ile Asp Ser Asp Cys Ile Cys Asp Cys Pro Gly Gly Asp Asp His
            100                 105                 110

Cys Ala Val Asp Tyr Ala Tyr Lys Asn Cys Thr Gly Asp Asn His Ser
        115                 120                 125

Ala Phe Cys Val His Thr Tyr His Pro His Gln Ser Ala Ala Gly Cys
    130                 135                 140

Gln Leu Ala Gly Glu Ala Asp Ile Cys Cys Lys Leu Val Val Lys Pro
145                 150                 155                 160
```

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```
Tyr Gln Asn Arg Arg Tyr Val Ala Val Gln Ile Gly Gln Pro Leu Thr
                165                 170                 175

Val Ala Thr Phe His Tyr Gln Leu Tyr Ser Lys Gln His Tyr Asp Gln
            180                 185                 190

Trp Gln Met Leu His Asp Gln Arg Phe Gln Ala Val Ser Gly Gln Ser
        195                 200                 205

Gln Arg Val Gln Leu Asp Gln Ala Gly Leu Gln Leu Gln Leu Gly Asp
    210                 215                 220

Leu Lys Pro Ile Trp Gln Leu Lys Glu Gly Met Tyr Val Phe Asp Leu
225                 230                 235                 240

Asn Asp Glu Asn Phe Thr Leu Arg His Gly Val Pro Ile Asn Arg Phe
                245                 250                 255

His Glu Tyr Ser Gly His Lys Leu Gly Trp Leu Arg Trp Gln Ser Ala
            260                 265                 270

Asp Lys Arg Trp Thr Val Arg Asn Gly Arg Ile Lys Leu Gln Ala Ala
        275                 280                 285

His Phe Val Gln Thr Val Asn Cys Leu Ala Gln Glu Tyr Ala Glu Thr
    290                 295                 300

Tyr Asn Ala Asp Phe Tyr Val Pro Ser Lys Glu Asn Gly Gln Lys Pro
305                 310                 315                 320

Phe Phe Leu Gly His Pro Val Glu Gln Thr Glu Arg Trp Ile Arg Thr
                325                 330                 335

Val Lys Leu Val Asp Arg Ser Ser Thr Ser Arg Gln Ile Gln Val Glu
            340                 345                 350

Gln Asn Gln Ser Pro Pro Ile Ser Ile Leu Leu Ser Phe Asn Ser Thr
        355                 360                 365

Val Gly Met Thr Val Leu Tyr His Gly Ser Glu Leu Gly Glu Phe Val
    370                 375                 380

Ala Thr Ile His Leu Asp Ala His Ser Asn Arg Phe Ile Asn Ile Thr
385                 390                 395                 400

Ala Met Asn Cys Lys Gly Thr Leu Ile Gly Gln Leu Tyr Arg Ser Asn
                405                 410                 415

Arg Gln Glu Thr Thr Glu Leu Val Phe Ser Ser Tyr Val Gly Gly Glu
            420                 425                 430

Asp Arg Leu Gln Asn Ser Thr Ile Arg Ile Gly Ala Pro Ala Thr Val
        435                 440                 445

Asn Gly Ser Arg Trp Leu Cys Leu Gln Pro Tyr Glu Lys Pro Lys Leu
    450                 455                 460

Gln Lys Cys Arg Trp Ala Thr Phe Ser Ala Gln Pro Leu Pro Lys Val
465                 470                 475                 480

Glu Leu Pro His Arg Trp Thr Gln Ala Gln Gly His Cys Thr Asp Cys
                485                 490                 495

Asn Gln Ile Ser Val Asn Asn Phe Leu Lys Tyr Leu Asn Pro Ala Asn
            500                 505                 510

Trp Thr Gln Gly Val Asn Gly Trp Ser Glu Ala Met Ala Val Gly Leu
        515                 520                 525

Glu Val Ala Phe Tyr Leu Leu Leu Ala Val Ile Leu Cys Ala Val Cys
    530                 535                 540

Arg Arg Leu Ile Cys Pro Val Leu Arg Trp Thr Ile Cys Gly Asn Gly
545                 550                 555                 560

Arg Thr Asn Ala Asn Lys Leu
                565
```

-continued

<210> SEQ ID NO 26
<211> LENGTH: 580
<212> TYPE: PRT
<213> ORGANISM: Branchiostoma floridae

<400> SEQUENCE: 26

Met Val Val Leu Arg Ala Val Leu Leu Trp Ala Ser Ile Ser Gly Ile
1 5 10 15

His Gly Asn Arg Cys Thr Lys Thr Leu Pro Thr Thr Gly His Val Arg
20 25 30

Tyr Ser Ser Arg Glu Ser Ala Gln Ala Leu Val Glu Thr Phe Ile Gln
35 40 45

Ser Ser Ile Lys Pro Gly Glu Thr Leu Cys Phe Thr Leu His Asp Val
50 55 60

Thr Asn Asp Asp Ala Thr Ser Asp Ala Ser Ile Ser Met Ala Thr Asn
65 70 75 80

Gly Ser Val Pro Leu Leu Trp Gln Leu Thr Tyr Glu Gly Ile Glu Met
85 90 95

Glu Tyr Gly Val Lys Asn Arg Tyr Ser Phe Tyr Arg Pro Lys Tyr Glu
100 105 110

Ser Lys Cys Ile Cys Asp Cys Pro Gln Tyr Gly Asp Tyr Cys Asn Ser
115 120 125

Lys Thr Asn Arg Cys Thr Glu His Glu Leu Asp Phe Cys Tyr Asn Thr
130 135 140

Tyr Arg Ser Asp Gln Thr Ala His Gly Cys Leu Ala Tyr Trp Asn Ser
145 150 155 160

Glu Glu Ser Glu Val Cys Cys Ala Leu Tyr Val Gly Lys His Pro Glu
165 170 175

Ser Pro Lys Tyr Asp Ala Val Tyr Leu Ser Ser Asp Gly Lys Pro Ile
180 185 190

Val Lys Leu Ala Leu Lys Val Tyr Asp Ser Arg Thr Asp Glu Val Val
195 200 205

Tyr Ser Tyr Pro Thr Phe Thr Val Ala Leu Asn Asp Arg Thr His Arg
210 215 220

Ala Gln His Ser Val Arg Met Glu Val Thr Gly Asp Thr Pro Thr Gln
225 230 235 240

Ile Asp Ser Gly Tyr Tyr Tyr Ala Thr Ser Glu Gly Thr Gln Leu Tyr
245 250 255

Thr Asp Val Ser Ile Asn Gly Leu Asn Glu Phe Asp Pro Arg Lys Met
260 265 270

Gly Trp Leu Lys Val Arg Asp Asp Gly Thr Val Glu Arg Pro Pro Glu
275 280 285

Arg Thr Val Leu Asp Ala Phe His Leu Lys Thr His Lys Cys His Asn
290 295 300

Lys Phe Asp Tyr Thr Glu Thr Trp Gln Ile Tyr Gly Glu Gly Asp Trp
305 310 315 320

Lys His Tyr Ser Gly Ser Arg Val Glu Asn Phe Tyr Gly Trp Val Arg
325 330 335

Arg Val Ser Tyr Tyr Pro Gln Arg Arg Ser Val Thr Val Val Pro Arg
340 345 350

Tyr Asp Arg Leu Val Thr Val Lys Ile Gly Ile Asn Thr Thr Thr Asn
355 360 365

Val Leu Phe Phe Tyr His Asp Ser Asp Leu Ile Asp Phe Thr Ala Glu
370 375 380

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```
Val Arg Val Asp Lys His Ser Asn Arg Phe Ala Asn Ile Thr Leu Val
385                 390                 395                 400

Ala Ala Val Gly Ser Leu Val Gly Ser Ile Thr Pro Tyr Tyr Gly Ala
                405                 410                 415

Asp Gly Ala Ser Ser Ala Thr Val His Arg Phe Glu Leu His Val Asp
            420                 425                 430

Ser Pro Pro Ala Ala Asn Thr Ile Lys Arg Ile Ser Leu Pro Lys Thr
        435                 440                 445

Ile Asn Gly Thr Ser Arg Met Cys Leu Ser Pro Leu Ser Lys Pro Asn
    450                 455                 460

Asn Glu Val Cys Lys Thr Val Pro Phe Ile Gln Glu Ala Leu Gln Asp
465                 470                 475                 480

Phe Phe Val Pro Pro Thr Trp Arg Pro Gly Asn Pro Gly Ser Ala Gly
                485                 490                 495

Pro Gly Phe Asn Phe Asn Trp Leu Phe Asp Phe Phe Gly Phe Leu Asn
            500                 505                 510

Pro Ala Glu Trp Phe Asp Gly Ile Gln Gly Trp Leu Glu Leu Phe Ala
        515                 520                 525

Met Leu Leu Asp Ile Ala Leu Phe Ile Ala Gly Ile Phe Leu Phe Ile
    530                 535                 540

Lys Val Cys Thr Cys Phe Asn Val Phe Thr Thr Lys Ala Pro Lys Trp
545                 550                 555                 560

Asp Glu Gly Val Glu Met Ser Val Leu Arg Arg Arg Lys Ala Glu Pro
                565                 570                 575

Gly Asp Val Cys
            580
```

<210> SEQ ID NO 27
<211> LENGTH: 1713
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide

<400> SEQUENCE: 27

```
atggtactgt ggcaatggtc aatagccgtt gccatatgtc ttgtgatggt tacagaagct     60

cgtctcagaa gacatcacag gaaacgacga tttgtatcta gtaattttga tgaatttttat    120

tgtggagaaa gtgcacatgc tcagtcacag tttgaagagg agcgagaatc aaattcctca    180

aaagtctcat cagtacacag tactcaattc aattggggac ttgataatac aatttgtata    240

aaactccaga atgtggttca tgttttaaaa tacgaacgcc tcgaacaaag atatcctatt    300

gaaaactcct acacattttc ggttccatta attgacacaa actgtaaatg tcattgttat    360

ggttttggga caaatgacgt ttgcaatgtg gaaaagtacg ctgacgacag aaattgtacg    420

acgagctcag agtttcctac atgttacacc aaatatcacc cagcagttga gccactagat    480

tgtccagtta caagtattcc tgccaaggca tgctgcgaca tcaagttaaa accacgcgat    540

ggcagaatgt tccgagctgt gaaacttcag caaccaatca atgacatgat aatttctcat    600

tctatttttg caaacaacag tggaaaaatg atgaaagttc tgggaccaga tgaatttagg    660

ataaatcttc tgaaggggaa ggaacaattt gagttaactg aataccacag aatatccgtt    720

caattagttg catcttctcc tcaacaacaa cttcgcgaag ggatgtatta ttttccagag    780

gaaaaccaca acgatctgcg tgagggtaaa attaatgaaa taactgaaag tgatttggat    840

aaacttggat ggtatagaag agttggaaat gattggcaag ttgctacaag tggattacta    900
```

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cttagaaatg cacataaagt ggtgataaaa aattgcaaag gacaagttca tatggatcaa    960

ttttccggaa ccaaaaactt tgttttacgt ggaactcagt ataacgatac ttacaatgaa   1020

cgaagagtat ctgacaataa ttttgtaaga agtgtgaaag ttgacgagtc ttctcgagaa   1080

attacaattg ttcatgaaca tggaaccgct gcacaagttt ctctgaaaac tgacactcgt   1140

ccaaatctaa caaaaagtca atcacttttg gcaaacttca ctggaagtat cacgttagat   1200

catgatggaa atcgaatgct taatgtcact ttctttggcg tcaaaggaac tgttcatatc   1260

aaaatgtatg tcaacgatcg aaagctcatc gcgacttttg catgtactgc tcaatttgga   1320

acgtctctga aagatgatgg tagtagaata agtcttccat cgactataaa tcaagctcaa   1380

tgggtgtgta ttcttcccga tgagcagcca acaaaatcag aaatatgcaa atggattcca   1440

tatgaggaaa aagcaatgag aactccgaga caagaacaaa gttggtcaaa aggacattca   1500

ccgtgctcac aagcagaatg taatagtctg aaaagtggag tgagcgactt gttcccatgg   1560

attatgaatt ttgattattt tatggctcat ggtggggatt tcacagagtg gctaaaaatt   1620

ggaatccatg ggcccttcga aggtaagcct atccctaacc ctctcctcgg tctcgattct   1680

acgcgtaccg gtcatcatca ccatcaccat tga                                1713
```

What we claim is:

1. A method for fusing a first mammalian cell and a second mammalian cell; comprising:

(i) incubating a first mammalian cell expressing a nematode fusogenic protein with a second mammalian cell expressing a nematode fusogenic protein; and

(ii) fusing the first and second mammalian cells, wherein the nematode fusogenic protein is selected from the group consisting of anchor-cell fusion failure 1 (AFF-1) and epithelial fusion failure 1 (EFF-1) and wherein the first and second mammalian cells are of the same species.

2. The method of claim **1**, wherein expression of the nematode fusogenic protein is transient or stable.

* * * * *